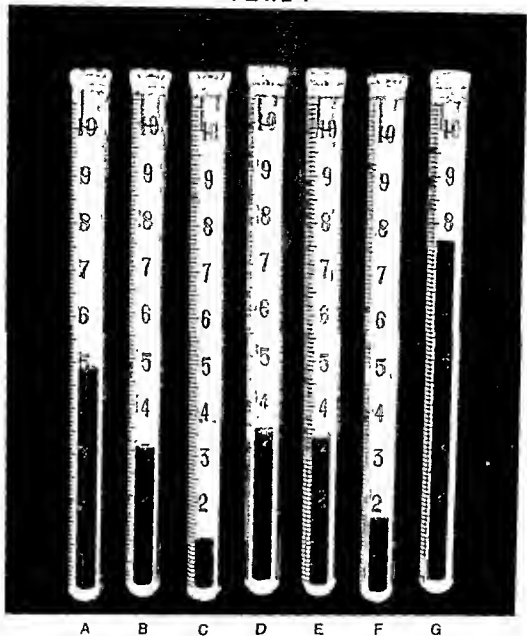


# Clinical Hematology

# PLATE I



*The appearance of centrifuged blood in various conditions*

A. Normal blood

B. Anemia associated with chronic infection

C. Iron-deficiency anemia The blood plasma is very pale

D. Chronic myelocytic leukemia There are distinct layers of white corpuscles and of platelets above the red corpuscles

E. Post hepatic jaundice and moderate anemia In this case the coloring of the blood plasma is due to biliar obstruction rather than to increased blood destruction

F. Pernicious anemia Note the small amount of packed red corpuscles, the very narrow layer of leukocytes and platelets, and the coloring of the blood plasma due to hyperbilirubinemia

G. Polycythemia

(The blood was collected and treated as described on page 110)



# Clinical Hematology

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To My Wife  
*and in memory of our son, Paul.*  
M.M.W.

# Preface

MORE than three decades have passed since I first began the task of assembling what was known about hematology in book form. Even then, the undertaking was not easy. Since that time, hematology has developed to a degree unmatched by any other field of medicine, in scope as well as in the depth of our understanding. Hematology now encompasses broad aspects of cytology, biochemistry, molecular biology, biophysics and immunology, not to speak of the various ramifications of clinical medicine.

The first six editions of *Clinical Hematology* were written entirely by me. With each edition the task has become more challenging and it has been more and more difficult to do justice to all dimensions of this remarkable field.

In planning the Seventh Edition, therefore, I enlisted the collaboration of some of my present and former associates. Their names are given on the title page. They were chosen because of their special interest in certain aspects of hematology, in addition to their thorough understanding of the whole field of hematology and their proved qualifications.

The organization of the book has been revised in keeping with present-day needs. In Part I, the approach to hematologic problems is considered from the viewpoint of the clinician. The principles of hematologic examinations are discussed and the simpler methods are outlined for the physician's own use. In addition, the principles and pitfalls of modern machines are presented. These discussions are presented so that the person who carries the ultimate responsibility for the patient will have enough understanding to do so with

confidence. This book, however, is not intended to be a laboratory manual.

In Part II a thorough account is given of the essentials of cytology, physiology, and biochemistry as they apply to the hematopoietic system. Such an understanding is essential for the intelligent and effective application of modern-day knowledge to medical practice. To attempt to practice hematology without an understanding of the normal hematopoietic system, as described in Part II, and without a physiologically basic concept regarding the approach to a hematologic problem would be like trying to sail in the open sea without landmarks or instruments and under a sky without stars.

To aid in the approach to the different varieties of hematologic problems, chapters and subsections which introduce the various areas or types of problems have been prepared; eg, the approach to the patient with anemia (Chapter 13), a kinetic approach to normocytic anemias (Chapter 19), an approach to hemolytic anemias (Chapter 20), a diagnostic approach to the bleeding disorders (Chapter 33), and the approach to the patient with disorders of the phagocytic and lymphatic systems (Chapter 40). In regard to treatment, the approaches are as diverse as hematologic conditions are varied.

In order to bring together in a single volume the ever-expanding body of information pertaining to clinical hematology, we have condensed some of the material found in earlier editions. Methodology, for example, has been given less space because advanced technology has produced sophisticated machines that have rendered it unnecessary for

the physician to carry out as many procedures as he used to perform. Yet he must understand these procedures—their purpose, their degree of reliability, and their pitfalls—and he must continue to be at home with elementary morphology, such as that required for examination of the blood smear and the bone marrow. Consequently, discussions of the latter topics have not been abbreviated. In hematology, morphologic examinations hold importance equal to the medical interview and the physical examination and should never be neglected. The morphologic descriptions are supported by 24 plates in color, most of which are new.

We have attempted to control the size of the book, but not at the expense of thoroughness and excellence. Furthermore, our original policy of providing, insofar as possible, careful and complete documentation, still obtains. As in past editions, reference lists are selective, as are citations in the text. When necessary, preference has been given to the latest report on a subject, in which the interested reader may find additional references to earlier literature. Whenever possible, comprehensive reviews and monographs have been listed. In citing the literature when a study has been carried out by more than two authors only the name of the first is given. Our apology is offered to those whose names are not listed.

In keeping with the decisions of the editorial boards of *Blood* and of the *British Journal of Haematology*, as well as other publications, we have adopted most of the units of the system recommended by the International Committee for Standardization in Hematology. These and their former equivalents appear inside the back cover of this book, as does our system of abbreviations. The style adopted for bibliographic material is that recommended by the *Index Medicus* of 1973.

Although each co-author, including the senior author, has assumed primary responsibility for certain chapters, no chapter is solely the work of one person, and all the material in this book has been thoroughly reviewed and edited by the senior author. Each member of the team has contributed in

some degree to chapters written by others, and all have utilized the material of earlier editions when this was appropriate.

As always, the preparation of a new edition of this book has been a rewarding though arduous task. Today's young hematologist probably has no conception of the rate at which his specialty has developed, especially since the mid-twenties. Clinical hematology exemplifies the ways in which clinical medicine has fed the basic sciences with questions and with clues, and the basic sciences in turn have illuminated human disease. The science of nutrition, for example, was enormously stimulated by the investigations that established the role of vitamin B<sub>12</sub> in pernicious anemia and those demonstrating the role of other vitamins in hematopoiesis. Exploration into the phenomenon of sickling opened the whole field of molecular biology and brought to light the many hemoglobinopathies of which we are now aware. These remarkable advances in turn prompted investigation of the pathogenesis of thalassemia, which revealed pathogenetic mechanisms hitherto unknown. Basic investigations of carbohydrate metabolism and of the metabolism of the red cell resulted in the recognition of enzyme deficiencies of the red cell, some of which have serious consequences. Many other examples of the interplay of basic science and clinical medicine could be cited.

We should like to express our gratitude to Dr. Arthur Haut of the University of Arkansas for two chapters; Dr. Wallace N. Jensen of George Washington University for one chapter; Dr. A. S. Wiener for reviewing the chapter on blood groups and blood transfusion and making valuable suggestions; Dr. Phaedon Fessas for valuable comments regarding the chapter on thalassemia; Dr. George Stamatoyannopoulos for his advice regarding hereditary polycythemia; Dr. W. A. Schroeder for his comments on hemoglobinopathies; and, for their comments and criticisms of various other chapters, Doctors E. J. Hershgold, Gerald Rothstein, R. E. Lynch, James Kushner, Dana Wilson and Joseph Sannella of the University of Utah, Phillip M. Allen, Byrd S. Leavell, Charles

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We are especially indebted to Dr. Albert Clarysse, formerly Assistant Professor of Medicine, University of Utah, for his skillful preparation of photomicrographs, as well as to Miss Anne Sasyniuk of the University of Manitoba; to those who generously furnished illustrations from their publications or personal collection; and to their publishers. These include Dr. Dorothea Zucker-Franklin, Dr. Walter Seggers, Dr. C. L. Conley, and Dr. William McDivitt.

Secretarial assistance has been provided by Vreni Bithell, Carolyn Bailes, Franklin White,

Georganna Barnes, Mary Welch, and Pearl Emery, but it is with very special appreciation that the many long hours of hard work and the extraordinary efficiency of the senior author's secretary, Mrs. Katharyn Rees, are acknowledged. Without such unstinting effort his task would have been much more difficult.

It is a special pleasure, also, to acknowledge the skill and whole-hearted cooperation of the publishers, Lea & Febiger and their staff, especially Mr. T. J. Colaiczzi and Miss Emily Anderson.

As in the past, I must express my sincere appreciation of the understanding and unselfish support of my wife. With this edition, the support of the wives of my collaborators also is gratefully acknowledged.

MAXWELL M. WINTROBE

*Salt Lake City, Utah*

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# Part I

## Introduction



## *The Approach to Hematologic Problems*

- General Considerations
- Principles of Hematologic Examination
  - Obtaining the Specimen
  - Anticoagulants
- Quantitative Analysis of the Formed Elements
  - Enumeration
    - Traditional Procedures
    - Electronic Methods
  - Multiple Parameter Automated Blood-Counting Machines
- Examination of Blood by Microscopy
  - Preparation of Blood Films
  - Staining
  - Special Histochemical Stains
  - Examination of the Wet Film of Blood
  - Electron Microscopy

### **General Considerations**

Certain diseases primarily affect the blood and blood-forming tissues and, even more frequently, disorders of other organ systems result in alterations in the blood. Consequently, broadly defined, hematologic problems are common and diverse. Clearly, therefore, the approach to a hematologic problem must begin with a thorough history and a complete physical examination. Without these, laboratory investigation in any amount cannot be very useful and can even be confusing.

In addition to the history and physical examination, it is necessary to measure the volume of packed red cells (VPRC, hemato-

crit), the concentration of leukocytes (WBC), and the differential leukocyte count, and to examine the stained blood smear under the microscope. The erythrocyte sedimentation rate may be added as a useful, nonspecific indicator of acute or chronic conditions. If a disorder affecting the blood is suspected, or if any abnormality is found in the aforementioned examinations and tests, additional studies may be indicated.

The point of departure in the hematologic evaluation generally is an objective observation of abnormality made during the physical examination (eg, pallor, lymphadenopathy, purpura, splenomegaly), or when the blood is examined (Fig. 1-1). The symptoms of hematologic disorders may be so varied and nonspecific that in themselves they may not suggest a hematologic problem. For example, unexplained fever, or extreme fatigability, may or may not be due to a hematologic disorder. However, a history of excessive bleeding under conditions which would not usually be expected to produce this symptom suggests a hematologic disorder.

The additional hematologic examinations which would be indicated if a hematologic disorder is suspected include measurement of the hemoglobin concentration, platelet count, reticulocyte and red blood cell counts, and calculation of the red blood cell indices. Thereafter, at each stage of the diagnostic process, other tests are selectively chosen according to the provisional diagnosis which

the physician may consider likely. In addition to the above-mentioned examinations, the tools available to the physician studying a hematologic problem include: (1) quantitative study of the bone marrow morphology; (2) microscopic anatomy by biopsy of the bone marrow, lymph nodes, liver, or other tissues; (3) radiographic anatomy of the chest, abdomen, or bones or other tissues as suggested by the examinations made up to that point; (4) bioassay and chemical assay of body tissues or fluids, including but not limited to plasma and urine; and (5) evaluation of physiologic function by following the fate of tracer substances. For tracing, normal metabolites such as iron may be labeled, or a nonphysiologic but noninjurious label may be attached to a normal substance or body constituent. A chromium-ion label for red cells or an iodine tag on transferrin are examples of the latter. Tracer methods require that the presence of the substance used as a label does not alter the normal physiology either qualitatively or quantitatively. Most often radioisotopes are used as the labels because of the relative ease of their subsequent identification and quantification. Sometimes the hazards accompanying these substances preclude their use in patients or in subjects of physiologic studies. The heavy isotopes which require mass spectrometry for their identification may sometimes be used in their place. Tracer studies have allowed great insight into normal and disordered physiology and in a number of situations are powerful tools for diagnosis.

Incisive points in the examination of the patient which especially relate to hematologic conditions and discussions of the various tools for diagnosis named above are presented in the chapters which focus on the problems identified by the various clues. Thus, examination of the bone marrow is discussed in Chapter 2. Techniques dealing with red cells in particular are discussed in Chapters 3 and 5. Techniques dealing with the study of granulocytes and monocytes are considered in Chapter 6, and those concerned with platelets and hemostasis and coagulation in Chapters 9 and 10. The approach to the study of

anemias in general is considered in Chapter 13, to specific types of anemia in Chapters 14, 16, 19 and 20, to problems of hemostasis and coagulation in Chapter 33, and to the patient with disorders of the phagocytic and lymphatic systems in Chapter 40. In the present chapter, only general principles concerned with the interpretation of hematologic data and certain details of hematologic technique are discussed because of their broad applicability to so much of that which follows.

It is important to stress that indiscriminate selection of a battery of hematologically oriented tests is not wise. Sometimes, however, when dealing with automated laboratories, it may be faster, no less accurate (depending upon the instrumentation), yet less costly in time and money to initially obtain a battery of hematologic data (to be discussed later) even for patients not suspected of having hematologic problems, in place of adherence to the selected items listed above. When utilizing some types of automated apparatus one may be led to forego the centrifugal determination of VPRC in patients without evident hematologic problems. However, when blood disease is suspected the VPRC should not be omitted.

Successful and intelligent management of disease requires a diagnosis or at least the best approach to a diagnosis. To achieve the diagnosis the physician engages in a deductive reasoning process. From the glimpses into the disordered physiology or anatomy of the diseased patient which are allowed by the assembled evidence, he must conceptualize the entire disease process. His hypothesis regarding the latter must include identification of those key points in the evolution of the disease which, if altered by therapy, would arrest or reverse the illness. Then, he must test his hypothesis by decisive action. The physician's role in this decision-making process requires that he be able to first answer the question, "What is the nature of the evidence which needs to be gathered?" and later, "Has all the relevant information been accumulated?" Uncertainty regarding these answers results in a diffuse testing and data-

# APPROACH TO HEMATOLOGIC DISORDERS

## Various Clues that Might Be Found

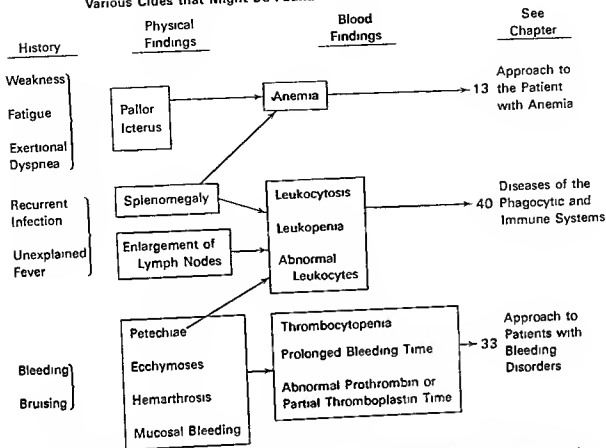


Fig. 1-1. This diagram relates sections of this book to clues from certain features in the *history*, *physical examination*, or *preliminary blood examination* which can guide the examiner toward the solution of a clinical problem

gathering exercise, which is disproportionately expensive and of limited if any value to the patient.

It is axiomatic that an understanding of the normal physiology of the blood, the bone marrow, the spleen, and the lymphatic tissues, and of the mechanisms of hemostasis is required for an orderly inquiry regarding the pathogenesis of derangements in these systems, and hence the pursuit of the diagnosis. Even an incomplete understanding of the disordered physiology provides some basis for management of those clinical problems which cannot be solved today. It also gives direction to investigative efforts which ultimately will provide the greatest relief for individuals suffering from as yet incurable problems in clinical hematology.

The diagnosis allows the physician to ap-

preciate the prognosis if one did not intervene with therapy. Knowledge of the prognosis, both with and without various modes of therapy, guides the physician in answering the three major questions of therapy:

*If he should treat,  
when he should treat,  
and with which modality?*

All too often, one encounters insufficient patience in attempting to achieve a diagnosis and undue haste in attempting therapy. The therapeutic measures, in particular the chemotherapeutic agents, available to hematologists in the present day carry a very substantial potential for doing harm. Potential gains must be weighed against potential risks.

The physician undertaking the management of a patient with a hematologic disorder



must possess more than a thorough understanding of normal physiology and of the normal course of the diseases with which he is dealing. He must also be mindful of the patient's fears and hopes and of those of his family. He must take the time to give the patient and his family some understanding of the illness, if there is one, and yet he must do this with sensitivity and sympathy. He must choose carefully each word he uses and must consider how his comments may be interpreted, or misinterpreted. The nature and course of certain hematologic disorders are such that in some patients reassurance may be far more important than any other measure the physician can offer. To undertake meaningless therapy, to treat only because of the magnitude of the white blood cell count, for example, without considering the psychologic effects of such attention to what may be a relatively minor manifestation of the disease, and to risk the possibility of injury by the therapeutic agents used, without considering the normal course of the disease in question, even when not treated, are errors of judgment which are all too common.

## Principles of Hematologic Examination

The correct interpretation of values obtained from examination of the blood depends equally upon a thorough knowledge of the normal values and of the *limitations or possible sources of error of the methodology* employed in generating the values for the specific patient. Often, the advantage afforded the hematologist in solving clinical problems lies as much in the care and precision practiced by the laboratory that he relies upon as in his own specialized knowledge. Small differences from the normal can allow important and valid inferences when laboratory technique is good; interpretation of even substantial differences from the normal may be hazardous when the methods relied upon are less reproducible, or less accurate, than those which we recommend. Carefully made observations and measurements in the laboratory, as in the physical examination, are the very

**Table 1-1. Sources of Error in Blood Cell Counts Introduced by Improper Handling of the Specimen\***

1	<b>INCORRECT SPECIMEN IDENTIFICATION</b> Blood drawn from wrong individual
2	<b>PROLONGED VENOUS STASIS PRIOR TO VENIPUNCTURE</b>
3	<b>EQUIPMENT NOT DRY</b>
4	<b>WRONG ANTICOAGULANT INCORRECT RATIO OF BLOOD TO ANTICOAGULANT</b>
5	<b>PRESENCE OF CLOTS</b> delayed or inadequate mixing too little blood hypercalcemia
6	<b>DILUTION ERROR</b> low accuracy or precision of pipet technician, or semi-automatic or automatic equipment

\*Applies to counts done by manual hemocytometer, and automated and electronic counter techniques

core of the most thoughtful analysis of a clinical problem. They begin as the blood sample is obtained. (Table 1-1).

### Obtaining the Specimen

Accurate and unequivocal labeling of the patient-source of the blood sample, and of the date it was secured, is essential. Small quantities of blood may be obtained by *puncture* of the lateral aspect of the distal phalanx of one of the patient's fingers, or, in infants, of the plantar surface of the heel. A sterilized (preferably disposable) styler or needle is used. Freely flowing blood obtained from these sites may be used for preparation of blood films, the platelet count, or for the microhematocrit method when venipuncture is not possible or is not preferred. Unless blood obtained for counting procedures is promptly mixed with suitable dry anticoagulants or diluents, agglomeration of platelets and other cells will occur, or fibrin strands or even a complete clot will form, and prevent reliable studies.

When several quantitative blood examinations are to be carried out, it is better to collect a few ml of blood from a vein. Suitably *anticoagulated*, this will allow dilutions and counts to be performed in a laboratory remote from the patient and at a later, more

convenient time. Although it is best to perform the counts within six hours after the blood has been drawn, counts will be satisfactory if the blood has been collected in EDTA and kept refrigerated for as long as 24 hours.<sup>27</sup> Several ml of venous blood are required if certain of the automated methods, to be described below, are to be employed for blood counting.

The venipuncture may be performed in one of the antecubital veins, with a sterile  $\#20$ ,  $1\frac{1}{2}$ " needle. One of the smaller veins of the forearm, or dorsum of the hand, may also be used, although if any of these sites is chosen a narrower needle ( $\#21$  or  $\#22$ , thin wall) is usually needed. In hospitalized patients, particularly in the obese, it is sometimes necessary to obtain blood from the femoral vein. The syringe and needle used to collect blood must be clean and dry; otherwise hemolysis will occur. Inexpensive needles, for single use, are commercially available. These are preferred by physicians and patients alike, because they are sterile and single use avoids the risk of transmission of homologous serum jaundice; they are sharp and free of burrs, thereby minimizing discomfort to the patient; and, ultimately, they are less costly than multiple-use needles which require hand processing for cleaning, sharpening, inspection, packaging, and sterilization following each use. Vacutainers are test tubes sealed under vacuum by a pliable stopper which may be punctured by the back end of a special needle held in its own adapter, after the forward end of the needle has been used to enter the vein in the usual fashion. With this device, the collection vessel also serves in place of the syringe.

After the blood is obtained, the tourniquet should be released before the needle is withdrawn. Direct pressure should be applied to the site of venipuncture to prevent hematoma formation. Several minutes of direct pressure will usually suffice, except in patients known or suspected of having thrombocytopenia or other derangements of the hemostatic system. In those individuals, at least five minutes may be required. The practice of flexing the patient's elbow with a small piece of gauze or

cotton in the antecubital fossa is often insufficient since pressure may be applied away from the point of puncture. It is better to elevate the patient's limb slightly and, with a piece of dry, sterile gauze or cotton over the puncture site, to apply pressure at the site with the thumb. It may be necessary to puncture these veins on many subsequent occasions, particularly in patients with significant illnesses. A few moments spent in the care of the vein after venipuncture will be returned manyfold through avoidance of frustration and discomfort on later occasions. Furthermore, if ecchymoses, hematomas, or other signs of technical problems develop at the venipuncture site, they may be readily interpreted by the patient as a true measure of the overall proficiency of the physician and his staff.

### Anticoagulants

An anticoagulant is required if venous blood is to be used for blood counting. The choice of anticoagulant and the ratio of blood to anticoagulant are of paramount importance. Dry anticoagulants are used to avoid dilution which would lower the concentration of the formed elements in the collected blood. The anticoagulant selected must not alter the size of the red cells, nor bring about their hemolysis; it must minimize aggregation of the platelets; it must minimize disruption of the leukocytes; and it must be readily soluble in blood. These qualities are best met by the dipotassium or tripotassium salt of ethylenediaminetetraacetic acid ( $K_2$ EDTA). The disodium salt ( $Na_2$ EDTA) is less quickly soluble but somewhat less expensive. Both of these salts are effective in the concentration of 1 mg/ml of blood. Greater concentrations may induce artifactual errors in the VPRC and other values.<sup>63,93,108</sup> Since a measured amount of dry anticoagulant is generally already present in the vessel used to receive the blood to be obtained by venipuncture, the operator must be certain that the volume of blood required ( $\pm 20\%$ ) for the amount of anticoagulant is actually

delivered into the vessel and promptly mixed with the anticoagulant. If too much blood is added, small clots may form; sometimes these are not easily noted but are, nevertheless, capable of altering the values. If too little blood is added, the red cells may be crenated, thereby falsely reducing the volume of packed red cells<sup>108</sup> and the corpuscular indices derived therefrom. Small bottles or other well-stoppered receptacles containing 3 or 5 mg of EDTA sufficient for 3 or 5 ml of blood, respectively, are popular. Small Vacutainers containing sufficient EDTA for 4 ml of blood have the advantage in that, when filled with a syringe pointing downward when its needle pierces the stopper, they have sufficient vacuum to assure their filling with the correct volume of blood, provided some excess blood remains in the syringe after the blood ceases to flow into them.

A mixture of dry ammonium oxalate and potassium oxalate, in the ratio of 3:2, is a satisfactory anticoagulant<sup>41</sup> which also avoids alteration in the volume of packed red cells.<sup>83</sup> Two mg of the mixture will serve to anticoagulate 1 ml of blood.<sup>41</sup> Either one of these oxalates used alone is inadequate, as the former causes red corpuscles to swell and the latter leads to shrinkage. The mixture, often called "balanced" or "double" oxalate, is more likely than EDTA to result in aggregation of platelets and, therefore, is less popular now as a general-purpose anticoagulant for hematology laboratories than formerly. However, double oxalate and not EDTA must be used as the anticoagulant for the "sucrose-hemolysis" test for paroxysmal nocturnal hemoglobinuria.<sup>84</sup>

Dry heparin may be used, but it is more costly than the double oxalate or EDTA and, since it may not mix readily or rapidly with blood, tiny clots may form. However, by comparison with blood from a hemophilic to which no anticoagulant need be added, it has been found that heparin does not alter the size of the corpuscular constituents of the blood. For this reason, heparinized blood has been used as a standard for comparison of the effects of various inorganic anticoagulants.

Since the formed elements of the blood tend to settle upon standing, and do so particularly in the presence of anemia, hyperfibrinogenemia, or hyperglobulinemia, it is essential that, prior to sampling for blood counting and other examinations, the venous blood be gently but thoroughly mixed. The specimen must also be inspected for macroscopically visible autoagglutination of red corpuscles and aggregation of platelets, and for the presence of clots. Insufficient attention paid to these simple matters may vitiate the significance of blood counts performed by the most costly robot apparatus, or by trained technologists.

#### Quantitative Analysis of the Formed Elements

Normally, and even in disease associated with severe anemia, the fraction of blood occupied by the red corpuscles is great enough to allow accurate quantitation of these cells simply by measuring their proportionate volume after centrifugation. The hematocrit described by Wintrobe<sup>175</sup> (Chapter 3) has the advantages of simplicity and high accuracy, and requires minimal technical skill. The Wintrobe tube may also be used to determine the erythrocyte sedimentation rate (page 125) prior to centrifugation of the sample, and afterwards to estimate the icterus index (page 214). The latter is a simple screening procedure to detect the possibility of hyperbilirubinemia. Normally the relative numbers of and volume occupied by leukocytes and platelets in the blood are too low to quantitate by measurement of the proportionate volume, which is about 0.5% to 1.0% of the total. Nevertheless, one can readily detect the presence of marked leukocytosis or thrombocytosis, or even leukopenia, by inspection of the reddish-gray (buffy) layer overlying the packed red cells in the Wintrobe hematocrit tube. This and other hematocrit techniques are discussed in Chapter 3.

The term "hematocrit" (G. *haima*, blood, + *kritē*, I separate) was at first used

to identify the *instrument* used to measure the proportionate volume of packed red cells (VPRC, PCV); ie, the particular type of centrifuge or the tube employed. Now, *hematocrit* is predominately used synonymously with the VPRC as determined by a centrifugal method. Unfortunately, the term "hematocrit" has also been applied to a value computed from measurements by automated devices of either the sizes of individual red cells<sup>40,66</sup> or the electrical conductivity of the blood.<sup>53,78,100</sup> Some investigators consider those values to be comparable to the VPRC.<sup>26,72,76,110</sup> Others,<sup>78,112</sup> including ourselves, do not. The computed values entail different principles than does the established Wintrobe method and they have unique sources of error as described later in this chapter. We will conform with current usage and employ the word hematocrit throughout this book to refer to all of these widely practiced methods of measurement. The term VPRC will be reserved for that value as determined by an acceptable centrifugal method (Chapter 3). VPRC will be used when specific differentiation from other methods is required.

## Enumeration

### Quantitation of Leukocytes and Platelets

This must be accomplished by enumerative procedures which measure the concentration of the leukocytes and platelets per unit volume, because of the small proportionate volume of these cells in the blood, and the irregularity of the relationship between their volume and numbers. Enumeration is also used to determine the concentration of red corpuscles ("red cell count") when that information is needed for the calculation of the corpuscular indices (Chapter 3). In both the traditional and the more recent automated procedures, two steps are required: (1) accurate dilution of the suspension to be studied, and (2) enumeration of the formed elements of interest, per unit volume of the diluted fluid.

## Traditional Procedures

The traditional procedure for blood counts has employed the Thoma<sup>145</sup> diluting pipet, or a variant of it. This consists of a capillary tube, graduated in relative units, containing a bulb in its upper portion. After blood is drawn to the mark in the capillary stem, diluent is drawn into the tube until the tube is filled to a mark in the short capillary stem above the bulb. The choice of dilution depends upon the concentration of particles to be enumerated. Ordinarily, a pipet allowing a dilution of 1:200 and 1:100 (v/v) is used for red cells, and another pipet which allows a dilution of 1:20 and 1:10 is used for leukocytes. A dilution of 1:100 is used for counting platelets except when thrombocytopenia is present, when a lesser dilution, such as 1:20, is employed. The correct diluent must be chosen according to the cells to be enumerated. For red cells, the diluent is an isotonic fluid such as Hayem's or Gowers' solution<sup>43</sup>; for leukocytes, Turk's diluting fluid is employed to hemolyze the usually more numerous red cells and stain the leukocytes; for platelets, the preferred diluent is 1% ammonium oxalate<sup>19</sup> with 0.1% to 0.44% EDTA, as this makes the red cells translucent under the phase contrast microscope, and the platelets assume a rounded shape<sup>130</sup> and are more readily recognized.

Unopettes are small, plastic reservoirs with flexible walls, containing a pre-measured ( $\pm 1\%$ ) volume of diluent suitable for a specified cell-counting procedure.<sup>171</sup> When filled with blood from a companion thin-walled glass capillary of uniform bore and specified length, the appropriate dilution of the blood specimen is achieved. Unopettes allow dilution with  $\pm 1\%$  to 2% accuracy if the recommended technique is followed, and individually are less costly than are the standard, blood-diluting pipets made of glass. However, as manufactured, Unopettes are not reusable; whether their disposable character results in a net saving in the cost of labor plus materials depends upon the individual laboratory. A modification to allow re-use has been described.<sup>32</sup>

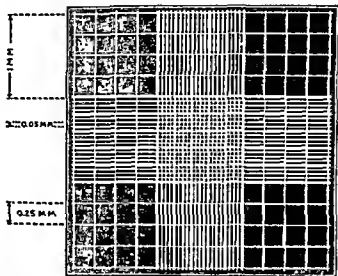


Fig. 1-2 \*Improved Neubauer ruling (1924) with double line surrounding each group of sixteen small squares used in all-glass chambers to produce the optical phenomenon of a single translucent boundary line

After thorough mixing in the pipet, the diluted blood is delivered into a *hemocytometer* (counting chamber). The latter consists of a heavy glass slide with two accurately ruled platforms, each surrounded by a moat and covered by an optically flat coverslip held exactly 0.1 mm above the platforms. The chamber now used is the product of many modifications.<sup>14b</sup> That of Neubauer<sup>14c</sup> is still employed (Fig. 1-2). After the cells have settled to the floor of the platform, those lying within specified ruled areas are counted with the aid of a microscope. Since the exact area enclosed by the rulings is known, as is the elevation of the coverslip over the platform, the volume of diluted fluid from which the enumerated cells had settled is also known. The latter would be strictly proportional to the area of the platform which was counted, if the assumption that all cells settled without any lateral shift were correct. Calculation of the concentration of the cells in the original sample then follows the formula:

$$\frac{\text{No. of cells}}{\text{Area}} \times \frac{1}{h} \times \text{Dilution} \times 10^6$$

= Concentration of cells in original sample

where,

Area = (mm)<sup>2</sup> enclosed by the rulings, as used in the particular counting procedure

No. of cells = the number of cells enumerated in the specified area

h = elevation in mm of the coverslip over the platform (usually 0.1 mm)

The specific multiplier factors pertaining to enumeration of red cells, white cells, and platelets are given in laboratory manuals<sup>11,49</sup> and earlier editions of this book.

Critique. Sources of error in the traditional hemocytometer technique are listed in Table 1-2. The assumptions that the cells in question are uniformly distributed per unit volume of diluted fluid in the pipet and that this homogeneity is not disturbed as the fluid is discharged through the narrow stem of the pipet and pulled under the coverslip of the counting chamber by capillary action are not strictly correct. Furthermore, as the cells settle in the counting chamber, some lateral displacement occurs. As a result, the cells enumerated in a given ruled area of the hemocytometer do not necessarily represent those from the overlying volume of fluid and thus an inherent error is created. This is true

**Table 1-2. Sources of Error in Blood Cell Counts Specific for Hemocytometer Technique\***

1. Inadequate shaking of pipet before filling counting chamber.
2. Failure to discard 4 drops from pipet before filling counting chamber.
3. Irregular filling of chamber trapped air bubbles, dust or oil on chamber or coverslip
4. Chamber coverslip not flat (especially important for phase-contrast method platelet counts with  $\approx 1\frac{1}{2}$  thickness coverslip)
5. Inaccurate rulings on chamber
6. Enumeration procedure Too many or too few cells included wrong borders, skipping cells, counting some cells twice
7. Total number of cells enumerated is too low to give statistical confidence in result
8. Error in recording from tally
9. Calculation error, failure to consider actual (non-standard) dilution, or area counted
10. Result entered opposite name of wrong patient

\*Also see Table 1-1

even if the diluting pipet, diluting technique, and hemocytometer rulings were all perfect, which, of course, they are not. If the inherent error of distribution of cells in the hemocytometer were a truly random process, then its effect could be reduced by increasing the sample enumerated, ie, increasing the area used and increasing the minimum number of cells counted in the sample.<sup>5,15,124,142</sup> Taking into consideration the inherent errors of calibration of the pipets and chambers and those of distribution,<sup>95</sup> a formula has been devised which indicates the probable minimum error in the procedure of counting red cells.<sup>6,7,124</sup>

$$C.V. = \pm \sqrt{\frac{8464}{c} + \frac{21.2}{h} + \frac{22.1}{p}}$$

where,

C.V. = one coefficient of variation (see page 19)

c = the number of cells enumerated

h = the number of hemocytometer chambers used

p = the number of pipets used

The formula also applies to enumeration of leukocytes and platelets, except that for the latter the constant in the numerator of the first term is  $10^4$ .

The number of cells counted in the range from 200 to 1000 has the most telling effect on the C.V. Beyond 1000 cells, there is little theoretical benefit from increasing the sample size, because the chamber and pipet errors become predominant.

If two pipets and two chambers are used and a minimum of 500 red cells enumerated, the inherent error results in a true answer lying within  $\pm 11\%$  of the reported result, on 95% of the occasions. If only 100 cells are enumerated (as in a blood sample from a patient with anemia or when a platelet or leukocyte count is made), the corresponding error is about  $\pm 22\%$ . In samples from a patient with anemia, leukopenia, or thrombocytopenia, fewer cells will be enumerated than would otherwise be the case, unless the technician makes compensatory changes in the technique. For example, a 1:10 dilution should be used in place of a 1:20 dilution for leukocyte counts, a 1:50, 1:20, or even lesser dilution for platelet counts; and a 1:100 dilution for red cell counts. A greater than usual area may need to be counted on the hemocytometer. Sometimes, changes in both dilution and area may need to be made.

Clearly, in addition to the above considerations, the technical proficiency of the individual who performs the actual dilutions and counting procedures is of the greatest importance. Idiosyncracies of technique<sup>125</sup> and subconscious bias in counting<sup>15</sup> result in greater reproducibility of blood counts for individual technicians without correspondingly greater accuracy of the results.

The error of a blood-counting procedure<sup>30</sup> which the clinician should normally accept depends upon the clinical implications of the different results created by such laboratory error. An error zone of even  $\pm 20\%$  (two coefficients of variation, 95% confidence limits) in leukocyte counts and platelet counts ordinarily does not have an important impact on clinical interpretation. Hence, hemocytom-

eter methods are acceptable for counting leukocytes and platelets. However, much greater precision is required for detecting and measuring anemia or polycythemia. The centrifuged VPRC and photoelectric hemoglobinometry (cyanmethemoglobin) have a considerably smaller error than that which may be achieved by counting red cells on a hemocytometer; hence, for the detection of anemia or polycythemia, centrifuged VPRC and photoelectric hemoglobinometry have supplanted the hemocytometer method of counting red cells. The red cell count is now primarily obtained for use as the divisor in calculating the corpuscular indices (MCV, MCH). In these calculations, an error zone greater than  $\pm 11\%$  could alter the interpretation of results significantly. Therefore, if the hemocytometer method must be used for counting red cells to determine the corpuscular indices, then painstaking technique (Chapter 3) should be followed.<sup>41</sup> However, electronic methods for enumeration of red cells are preferred since they have the capacity to reduce the error considerably below that of the traditional hemocytometer techniques, even to  $\pm 2\%$ .<sup>21,26</sup> At present, with due regard for technical matters, the most accurate measurements of red cell indices can be achieved by the use of electronic red cell counting (as discussed below), photoelectric hemoglobinometry by the cyanmethemoglobin method, and the determination of the VPRC by the Winrobe macrohematocrit method.

*The examination of a well-prepared, well-stained blood smear by the physician himself when a patient is suspected of having a hematologic disorder is just as important as the physical examination of the patient. This will not only provide important diagnostic information, but it is the physician's best safeguard against laboratory errors. When the experienced physician's interpretation of the stained blood film (page 27) conflicts with the information reported by blood-counting procedures, the wisest course is to have another blood film made and stained, and also to repeat the blood counts, rather than accept one and disregard the other. Not only may*

technical factors lead to error of either interpretation or counting, but there is always the chance of mislabeling of specimens. When properly performed, both blood counts and stained blood films should yield results that agree with and supplement each other. Persistent conflict of data from the two methods must be explained. Error may be one explanation, but may not necessarily be responsible. For example, the interpretation of hypochromia on the blood smear which is not supported by a low MCHC, could be explained by the presence of leptocytes (Chapter 26).

### Electronic Methods

Electronic and automated procedures for enumerating the formed elements in the blood have several advantages over the hemocytometer techniques. Chief among them are a greater precision (reproducibility), reflected in a lower coefficient of variation, and the capacity for completing a large number of determinations quickly and without increasing error due to fatigue. However, the cost of the electronic cell-counting instruments runs into many thousands of dollars (\$2500 to over \$50,000), depending in part upon the numbers of samples processed per hour, the degree of automation, and the number of ancillary measurements performed. Accordingly, these instruments are mainly employed in laboratories where a large number of determinations are to be made each day and where economic factors weigh in their favor. They are also popular in laboratories engaged in research or in clinics requiring the greater precision of blood counts achieved by these instruments when they are properly adjusted and calibrated. Some instruments can also measure cell-size distribution<sup>22,75,121,170</sup> and do so with an accuracy and speed which far surpass the now obsolete manual and visual methods.<sup>94,147</sup> In addition they have also been adapted to quantitate hemagglutination reactions.<sup>16,78,80</sup>

Several different instruments are available and are widely accepted. Almost all employ

transducers which depend upon either the *impedance principle*, or the *dark-field optical principle* for enumerating cells.

### Impedance Principle

This is used in the Celscope, Coulter, and the General Science Corporation Haema-Count systems, to name a few. A regulated constant current is passed between two platinum electrodes immersed in a special, electrically conductive diluent which is selected according to the cell type to be counted. The electrodes are isolated from each other (Fig. 1-3) by an insulator containing a small aperture (60 to 100  $\mu\text{m}$ ). The cells to be enumerated are first diluted with great accuracy and they must be evenly suspended in the diluent fluid. As the suspension is drawn through the aperture, each cell displaces its own volume of the conducting electrolyte solution within the cylindrical aperture. The cells are poorer conductors than the solution. As they pass through the aper-

ture, they individually produce a measurable change in impedance<sup>47</sup> which is very nearly proportionate to their individual volumes.<sup>105</sup> The resulting electrical pulses are amplified and are counted during the time an accurately metered volume of the suspension is drawn through the aperture. Most impulses of "less than threshold value" are attributed to electronic noise, and are rejected after discrimination.

In addition to the particle count per unit volume, a number of other values may be computed electronically from the same measuring process. By coupling the counting instrument to an electronic device for sorting pulses by their amplitude and storing these in a series of registers (multi-channel analyzer), the *cell-size distribution* may be computed, and tabulated or automatically graphed by an X-Y recorder.<sup>22,121,128</sup> Pulse-amplitude summation may be recorded simultaneously with pulse enumeration, and, when the former is divided by the latter electrically, this yields the average pulse size (ie,

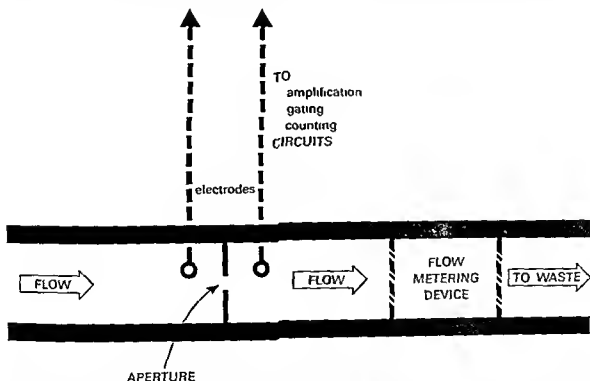


Fig. 1-3. Schematic diagram of the impedance type of electronic cell counter (see text, this page). The diluted sample containing the suspended cells moves through the aperture. The impedance is measured by the electrodes. It changes abruptly with each particle that passes through the aperture. The electronic circuitry counts the pulses in relationship to the flow of a metered volume.



mean cell volume, MCV). Furthermore, by taking advantage of the relationship

$$MCV = VPRC \div \text{Number of red cells (RBC)}$$

it is apparent that the "hematocrit" can be computed as the product of the MCV and the number of red cells<sup>10</sup>; and, that the "hematocrit" would be proportionate to the pulse-amplitude summation. Thus, in addition to enumerating white cells, platelets, or red cells, this type of equipment can electrically compute a simulated "hematocrit" and the MCV (Chapter 3) in less than a minute, almost simultaneously with the enumeration, and do so without employing a centrifuge.<sup>40-46</sup> The sources of error in the method are discussed later in this chapter.

A capacitance type of counter (TOA) is similar to the impedance types except that the electrical signal is developed by the change in capacitance resulting from the passage of a particle through the aperture.<sup>52</sup> However, with this instrument the electrical signal is not proportional to the cell size<sup>53</sup> and therefore cannot be used for cell sizing, volume distribution, or electronic simulation of MCV or hematocrit.

### Darkfield Optical Principle<sup>107</sup>

This is used in the Fisher Autocytometer, Technicon Autoanalyzer, and in SMA-4, SMA-7, and Hemolog electronic instruments.

A narrow stream of cells, uniformly suspended in an appropriate diluent, passes the focal point of a microscope-type objective in single file, in a darkfield illumination system<sup>17</sup> (Fig. 1-4). Each cell causes diffraction and forward scattering of the light and the latter is carried by a series of mirrors and lenses to a photomultiplier tube.<sup>126</sup> The image of each cell, falling one at a time upon the tube, alters it from the dark to the illuminated state and thereby generates an electrical (voltage) pulse. The pulse is amplified and counted, in relationship to the delivery of a known volume of diluted cell suspension, as it passes through the counting channel. The pulses in this system are not sufficiently proportional to cell size to readily allow the MCV or the "hematocrit" to be electronically represented from the enumeration data alone. Sources of error in this type of system are discussed below.

Double-scan instruments are a variation of the darkfield type. However, they move a chamber filled with a fixed volume of diluted blood to-and-fro and sideways by means of cams, rather than count a flowing stream of cells.<sup>122</sup> In this design, the slit aperture sweeps over an area of the chamber determined by the number and length of its excursions, the optical magnification, and the slit dimensions. Cells which lie only partially within the field of view, and therefore produce too small a signal to be detected, are

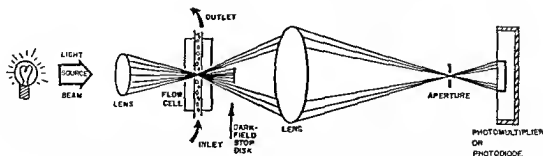


Fig 1-4. Schematic diagram of the operating principle of the darkfield-optical type of electronic blood cell counter. Light from a source lamp is passed through an aperture (not shown) and is focused at the center of the narrow stream in the flow-cell. When a blood cell is not at the focal point, light passing through the cell is stopped at the darkfield disk. When a blood cell is present, light is scattered so that it passes around the disk as illustrated by the three most lateral rays, above and below. The scattered light is collected by a lens and projected through an aperture to a photomultiplier or photodiode light detector. Each cell creates a pulse of light on the detector (see text).

differentiated from background by a second scan at altered slit dimension.<sup>48</sup> The need to fill a chamber with a static volume and the mechanical requirements for chamber movement lend themselves less well to high productivity than do the previously mentioned types of equipment.

USES. The electronic counters may be used to count *leukocytes*<sup>2,70,71,80,141,150</sup> and *platelets*<sup>35,37,54,59,60,68,82,160</sup> if particular adjustments are made in the instrument settings for threshold, aperture current, and signal amplification. The choice of diluent is critical for leukocyte work in order to eliminate interference from red cells; this is discussed below. To count platelets with the impedance type of instruments, interference from red cells is avoided by a preliminary sedimentation step so that only the plasma is diluted and counted.<sup>37</sup> With the darkfield optical type of instrument, platelet counts may be made on whole blood by rendering the red cells of the same refractive index as plasma through the addition of urea solution to the stream of cells prior to entry into the darkfield. Leukocytes are differentiated from platelets by threshold setting and their number is subtracted from the total.<sup>29</sup>

CRITIQUE. The technical superiority of the electronic instruments in enumerating cells of any given sample depends largely upon a far greater sample size than is possible with the hemocytometer. A technician using a hemocytometer might enumerate 500 red cells in a chamber in about five minutes. The electronic counter would enumerate about 50,000 cells from such a specimen in less than half a minute. Counts may be repeated with a C.V.  $\pm 1$  to 2%.<sup>21,128</sup> To reduce the C.V. on a single blood sample to about  $\pm 1\%$  with the hemocytometer technique would require the enumeration of about 50,000 cells and the use of 50 pipets and 50 chambers—a full day's work for one technologist. Avoidance of the error of distribution on a chamber and of the variation in volume from one chamber to the next, accuracy of recording during the actual counting process, and stable performance without fatigue are additional factors favoring the electronic counter.

**Table 1-3. Sources of Error in Blood Cell Counts Specific for Electronic Counters\***

- 1 **INCORRECT DILUENT OR LYSIS AGENT†** for particular instrument
- 2 **EXTRANEOUS PARTICLES IN DILUTING FLUID** (or containers, at any step)
- 3 **PRESENCE OF CELL TYPE WHICH WAS NOT TO BE COUNTED**
- 4 **DESTRUCTION OF CELL TYPE WHICH WAS TO BE COUNTED**
- 5 **ERROR IN METERED DELIVERY OF CELLS AFTER DILUTION** pump, valves, tubing, connections cut-off switch
- 6 **PARTIAL OBSTRUCTION OF APERTURE** (impedance type instrument)
- 7 **COINCIDENCE LOSS**
- 8 **THRESHOLD SETTING** sensitivity or potentiometer setting not determined by proper calibration
- 9 **CARRY-OVER** from one specimen to the next
- 10 **SPURIOUS PULSES FROM SENSING REGION** of equipment due to air bubbles
- 11 **SPURIOUS SIGNALS** from electrical or RF interference
- 12 **INSTABILITY** or intermittent failure of electronic components

\*See also Table 1-1

†Applies to leukocyte and platelet counts

It is often assumed that great accuracy and reproducible performance are inherent properties of sophisticated electronic devices. As a result there is a strong inclination for physicians to accept the results of electronic cell counters as being accurate and beyond question. Not only are those assumptions false, for electronic performance varies with design and component quality, but, in the instance of cell counters, there are considerations beyond the electronic system which have significant bearing on the results. The possibility of electronic malfunction can be evaluated with a simple device.<sup>169</sup> Other potential sources of error in the use of electronic counters are listed in Table 1-3.

Failure to observe all of the precautions mentioned earlier when obtaining the speci-

men of blood could create gross error in the numerical output. Unfortunately, misleading or ambiguous labeling of the patient source, or of the sample number, does occur. This possibility must be kept in mind when the machine output does not correspond with the clinical situation.

Devices for counting particles electronically require greater dilution of the blood than does the hemocytometer technique. Obviously, inadequate precision in making the dilution can negate the accuracy that might have been achieved by the sophisticated device. The method for making the dilution may be critical to the reliability of the overall results. Either micropipets, calibrated within  $\pm 1\%$  error, or commercially available low-cost capillaries which fill automatically with an accuracy of  $\pm 1.25\%$  may be used in conjunction with pre-dispensed diluent. Properly calibrated semi-automatic dilutors<sup>38</sup> (which are moderately expensive) are another alternative to provide the accuracy warranted in the dilution step for electronic cell counters. In the Coulter Model S the dilution system is an integral part of the apparatus. The Hema-Aliquanter is said to be more accurate than standard pipets and less costly than other semi-automatic dilutors.<sup>39</sup>

In the Coulter counting systems, an error in diluting will not only create an error in the enumerated red cell count, but also it will produce an error in the same direction in the "hematocrit" which is computed by the automatic accessory for the Models B, D, and F, or the Model S; and in the MCV, if that is calculated by the technician from the electronic red cell count and the centrifugal VPRC.<sup>40,46</sup> However, the MCV which is computed automatically by the Model S, or the accessory for the Models B, D, or F, is not affected by erroneous dilution, although it is influenced by the threshold setting.

Leaky valves or other faults in the metering system which delivers the diluted samples to the sensing region can produce erroneous results, since standardized counting volume (or flow rate, at fixed counting time) is required.

The choice of diluent is also important.

For red cell counts an isotonic solution is needed so as not to alter cell volume. Isotonic saline alone is satisfactory if only counting is done.<sup>21</sup> If cell volumes, MCV, or "hematocrit" is to be automatically computed or cell sizing is to be done, a buffered medium is needed to avoid notable swelling of cells<sup>45,115,137</sup> which otherwise will occur with time.<sup>22</sup> A solution consisting of 0.17 M NaCl and 0.3 M tris(hydroxymethyl)aminomethane (Tris, THAM) buffered to pH 7.4 with HCl, 95:5 (v/v) is perfectly satisfactory.<sup>46</sup> It is easier to prepare and keeps better than does the more elaborate Eagle's salt-dextrose medium. The latter had been shown to give a stable cell volume-distribution curve, very similar to that of red cells suspended in plasma,<sup>22</sup> and had been widely used for that purpose. The solutions recommended by the instrument manufacturers (eg, Isoton) are also satisfactory and have the advantage of being filtered by the manufacturer to remove microscopic particles.

Selection of a diluent for counting leukocytes is very critical. Red cells must be lysed to avoid interfering counts. If destruction of leukocytes occurs in addition to lysis of red cells, spuriously low leukocyte counts result. Erroneous high counts may be produced if there are residual erythrocytes or stroma particles. Furthermore, with some diluents the value of the machine count varies with the interval after the dilution is made. Triton-X 100,<sup>2</sup> saponin,<sup>150</sup> 0.1 N hydrochloric acid,<sup>142</sup> and cetyltrimethylammonium bromide (Cetrimide, B.P.; Cetavlon<sup>TM</sup>)<sup>85,166</sup> have been advised as diluents which also lyse erythrocytes. Cetrimide with acetic acid had been preferred<sup>144</sup> because its stromatolytic action is much more rapid and more complete than that of saponin. The latter is totally unsatisfactory for mouse blood.

Cetrimide-citrate-saline with acetic acid<sup>50</sup> is even better because the addition of citrate abolishes the cytolytic effects of cetrimide on leukocytes and allows constant leukocyte counts on electronic instruments for as long as 24 hours after the dilution is made. However, occasional loss of lymphocytes occurs in blood from chronic lymphocytic leu-

kemia,<sup>138</sup> giving a falsely lower leukocyte count.<sup>57</sup> Substitution of ethylhexadecyldimethylammonium bromide (EHDA) (Eastman Kodak, Rochester, N.Y.) for cetyltrimethylammonium bromide in the citrate-saline, acetic acid formula<sup>70</sup> produced as good or better results with both the Coulter Model B and Fisher Autocytometer, and we recommend it for leukocyte counting. In chronic lymphocytic leukemia, the results compared well with hemocytometer techniques and loss of cells was not a problem.

Normally, a 1:500 dilution is made. When leukopenia is suspected or when fewer than 4000 leukocytes are enumerated in the volume (usually 0.5 ml) of blood which is counted at a 1:500 dilution, a 1:100 dilution is made by using a more concentrated ("low-count") diluent. The additional EHDA detergent in the latter formulation assures complete lysis of the additional quantity of red cells. The same solution is used, for the same reason, when counting polycythemic blood.

Of course, all diluents must be free of *extraneous particles* prior to use.<sup>52</sup> A simple filtration apparatus with triacetate filters such as the Millipore or Mettracel type with 0.45  $\mu$ m pores should be used. Commercial, suitably filtered diluent may be obtained from the instrument manufacturers.

*Coincidence loss* occurs when two cells enter the sensing zone of the apparatus simultaneously, or the second enters immediately after the first but in the brief refractory period as the signal from the first is developed. In both instances, only one cell is counted. In the impedance type of instruments, if cell sizing is being done, one large cell is recorded in place of two smaller cells. This error is a direct function of the concentration of cells in the diluent and the volume of the sensing region of the apparatus.<sup>128</sup> Greater dilutions reduce the problem, as do apertures of smaller diameter (60  $\mu$ m vs 100  $\mu$ m) and depth. Correction tables are supplied by manufacturers and these must be used to compensate for coincidence loss, unless the apparatus does so automatically, as, for example, the Coulter Model S and the

Fisher Autocytometer. Artifacts of cell size and volume distribution curves are related to aperture dimensions,<sup>31</sup> but asymmetry of volume distribution curves may describe a real phenomenon.<sup>3,120,171</sup> Antibody-coated cells also result in spurious macrocytosis.<sup>25</sup>

A small amount of diluent containing cells remains in the system after each count. This *carry-over* may be more significant in the Fisher Autocytometer, Technicon Autoanalyzer, and SMA series than in other instruments, and in Coulter Model S than in other Coulter models. Therefore, when the results of two successive samples have widely separated values, particularly in leukocyte counts where values of abnormal samples might vary by as much as a factor of 100 or more from each other, it is best to repeat the second count to ascertain its correct value. Normally, leukocytes are so infrequent compared with red cells that their presence may be disregarded when performing a red cell count. In leukemia, when leukocytosis and anemia are both present, a correction may need to be made.

It is clear that, in all electronic counters, some signals may be generated from the apparatus itself ("electrical noise") and that a highly favorable ratio of true *signal to noise* is needed for accurate counting. Low-amplitude noise can be discounted with high efficiency by discriminator circuits, if the instrument is electronically stable. Random entry of extraneous large signals sometimes results from sudden line-voltage fluctuations, as when other apparatus or electric motors are turned on and off. Electrical isolation transformers and voltage regulation devices may be needed. Radio-frequency pulses (RF) from electrical apparatus, flickering fluorescent lamps, and sparking motor brushes may sometimes be detected as "counts" by these counters. Additional RF shielding may be required to eliminate them. Inspection of the oscilloscope screen on the Coulter usually, but not always, indicates the occurrence of RF interference.

Setting the lower *threshold* eliminates spurious electronic and RF pulses of low amplitude and can also be used to reject

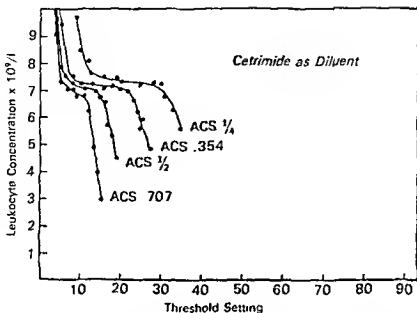


Fig 1-5 The relationship of the 'threshold' setting value to the 'count' enumerated by a Coulter Counter. Clearly a wide variance in the 'count' can be obtained depending on the value selected. The low slope of the 'plateau' represents the small-size end of the distribution of cell sizes. Threshold settings are usually chosen in this region, characterized by the smallest variance in 'count' for a given error in adjustment, although there is no assurance of 'accuracy' by taking this approach. The placement of the curve is a function of the aperture current setting (ACS) and amplification. (From Gagon et al,<sup>70</sup> courtesy of authors and Williams & Wilkins Co)

pulses from particles smaller than those of primary interest. For example, platelets may be disregarded when counting the larger red cells simply because they are so much smaller in size. However, if the threshold is raised too high (for example, in the Coulter Model A or B), the smaller red cells would not be included and a falsely low red cell count would result (Fig. 1-5). If the MCV were calculated from that value, it would be falsely high. This error is most likely to occur when very microcytic red cells are counted, as in severe iron-deficiency anemia, even though perfectly satisfactory results might be obtained when counting red cells whose MCV and cell size or distribution were normal or greater than normal. In fact, by setting the threshold value higher or lower, one can generate almost any value<sup>73</sup> for the instrument's "red cell count." Since cells vary in size even in healthy persons, in a nearly gaussian distribution in the case of red cells,<sup>22,34</sup> raising the threshold of a Coulter counter can pro-

gressively lower the count by excluding cells.<sup>73</sup> Lowering the threshold can spuriously raise the count by including non-red cell particles and even the electrical noise impulses.

Calibration of the instrument is therefore of prime importance and relates to threshold values and discriminator and potentiometer settings, even if the latter were set at the factory. Since, with variation of the potentiometer settings, virtually any printed output can be produced on the Coulter Model S, this instrument, as well as the others, must be calibrated by the user<sup>24,36,117</sup> to assure its accuracy. Accuracy is the quality that describes proximity of a measurement to the "true" or "real" value of the measured item. Reproducibility, or precision, is the measure of the closeness together of measurements of the same item upon replicate determinations. An incorrectly calibrated instrument, otherwise functioning well and with high precision, would yield the same wrong answer

every time a replicate of a sample was tested. The user must not mistake precision for accuracy. The precision usually reported at  $\pm 1$  or 2 C.V. represents the usual (minimum) error range to be expected from isolated determinations, even if the mean of a series of replicates were exactly the "true" value. One coefficient of variation (C.V.) is the ratio of the standard deviation (S.D.) to the mean ( $\bar{X}$ ), expressed as a percent. That is, C.V. (%) =  $[(S.D.) \div \bar{X}] 100$ . For example, precision of  $\pm 2\%$  (2 C.V.) means that 95% of the time the reported value will be not more than  $\pm 2\%$  away from the true value. Stated in another way, 95% of the time the true value would lie within  $\pm 2\%$  of the reported value. Statements of instrument performance or data often appear in journals, books, and advertising as  $A \pm b$ , without identifying whether "b" signifies  $\pm 1$  S.D.,  $\pm 2$  S.D.,  $\pm 1$  S.E.,  $\pm 2$  S.E., or some other value. Such statements are too vague to be meaningful<sup>62</sup> but are all too common in the literature of electronic counters.

Quality-control specimens must be run on each instrument under operational conditions<sup>58,117,167</sup> to assure continuing satisfactory performance. Unfortunately, an absolute reference standard is lacking for enumerative procedures.<sup>64,105,135</sup> To obtain the "true" value on a sample of blood, recourse must ultimately be had to many replicate determinations by hemocytometer techniques, which, paradoxically, are less precise than the electronic method one wishes to calibrate.<sup>24</sup> Since there is a 95% probability that the true mean lies within  $\pm 2$  S.E. of the experimental mean, and the S.E. =  $S.D. \div (N-1)^{1/2}$  (where "S.D." is the standard deviation of a series of measurements and "N" is the number of measurements in the series), it becomes possible to define the true value for cell counts, within about  $\pm 2\%$ , by performing a series of about 17 to 26 measurements. Laboratory reference standards which are prepared from blood and are stable for 14 to 28 days may be used for daily standardization of automatic instruments. These are available commercially, or may be prepared from formalin-<sup>132</sup> or glutaraldehyde-treated red cells,<sup>116</sup> or by

processing blood by a method incorporating adenine, citrate, phosphate, dextrose, hydrocortisone, and promethazine.<sup>177</sup> Standards for leukocytes<sup>113</sup> and platelets<sup>134</sup> have been described. Refrigerated specimens may be carried forward daily as another type of verification.<sup>27</sup>

### *Multiple Parameter Automated Blood-Counting Machines*

#### *Principles*

Automation of the most commonly requested hematologic blood tests has been combined into single instruments to conserve time and labor and reduce costs in laboratories with a large volume of daily work. Widely used in large hospital laboratories are the Coulter Model S<sup>TM</sup> and Technicon SMA-7A<sup>TM</sup>. These combine automatic sample dilution with their individual type of red and white cell counting apparatus described above, and also perform hemoglobinometry photoelectrically as cyanmethemoglobin.<sup>24,71,76,103,136,157,159</sup> A computed value for "hematocrit" is produced by the Coulter instrument, and the red cell indices are further computed. Digital output for hemoglobin concentration, "hematocrit," RBC, WBC, MCV, MCH, MCHC is produced at the rate of two to three samples per minute. The SMA-7A performs the same measurements, except it estimates "hematocrit" from the electrical conductivity of the whole blood (conductivity cell volume, CCV) and computes the red cell indices with that value. The Fisher Hem-Alyzer<sup>TM</sup> measures the red and white blood cells by using the dark field-light scattering principle, and measures hemoglobin as cyanmethemoglobin.<sup>111</sup> "Hematocrit" is not attempted and red cell indices are not calculated. The Technicon Corporation's Hemolog<sup>TM</sup> performs blood counts and the CCV as does the SMA-7A. It also does a platelet count<sup>29</sup> and adds a centrifuge device continuously operating at 23,000X G. In the latter, the packed cell-plasma interface is detected photoelectrically, and followed mechanically to esti-

mate the "hematocrit."<sup>149</sup> Other parts of this instrument measure the prothrombin time and the partial thromboplastin time.

**CRITIQUE.** In general, good precision in cell counting and hemoglobinometry (CV,  $\pm 1-2\%$ ) is achieved by combining automatic dilution with efficient mensuration. Carry-over of 2 to 7% in the Coulter Model S may produce 5%<sup>24</sup> or even 25% error when a true sample value is less than one third of its counterpart in the immediately preceding sample. For example, in one published report, when a sample with a WBC of  $8.4 \times 10^9/l$  was followed by one of  $1.5 \times 10^9/l$ , the instrument reported  $2.0 \times 10^9/l$  for the latter.<sup>157</sup> Unless the operator is alert for such sequences and runs the low-count sample again, day-to-day precision and accuracy will be adversely affected in certain patients with low blood counts, whereas performance of the instrument will remain good in other patients. Leukocyte counts greater than  $50.0 \times 10^9/l$  interfere with hemoglobinometry and are rejected by the instrument. Measurement of the MCV and "hematocrit" are good with the Coulter Model S and are not adversely affected by too low a ratio of blood to dry anticoagulant in the sample.<sup>28</sup> As in the case of the attachments for the Coulter Models B and F, the computed MCV is independent of dilution error, but is susceptible to error in setting the lower threshold and instrument calibration.

The "hematocrit" as computed by the SMA-7A is also termed the *CCV* (conductivity cell volume). This is based on the principle—published in 1898<sup>31,162</sup>—that the electrical conductivity of the blood is an inverse function of the volume of the packed cells.<sup>66,67,74,161</sup> Refinements in technique<sup>100,127</sup> and engineering have produced an instrument which performs well with normal blood.<sup>77,100</sup> However, erroneously low values result when insufficient blood from venipuncture is put into the collection tube, giving a ratio of dry anticoagulant to blood exceeding 1.0 mg EDTA/ml blood.<sup>112</sup> Furthermore, calibration which is performed with two concentrations of potassium chloride has been reported to be non-linear,<sup>100</sup>

and abnormalities in serum proteins and electrolytes, such as those which occur in patients with significant illnesses, introduce additional error to the technique, as do lipemia and marked leukocytosis.<sup>53</sup> Consequently, in certain samples the reported "hematocrit" (CCV) and the machine-calculated MCV may be erroneously low, whereas the MCHC will be erroneously high.<sup>75,76</sup> The physician must be particularly alert in interpreting such data from this instrument, because diagnostic conclusions run the risk of error. The Hemalog™, manufactured by the same company that produces the SMA-7A, has an automatic centrifugal hematocrit in addition to the CCV device and other features of the SMA-7A. An independent appraisal of the performance of this augmented instrument has not been published.

Differential leukocyte counts can be made by machine by three different methods. These are (1) analysis of cell size distribution; (2) computerized pattern-recognition of stained cells; and (3) analysis of relative light scattering and light absorption by leukocytes after histochemical reactions.

Reliable differentiation of lymphocytes from neutrophils and monocytes, and also from erythrocytes, has been accomplished in dilute suspensions by a multiple-channel analysis of the size distribution among 100,000 cells, as determined by an impedance type of electronic particle counter.<sup>139,176</sup> The preliminary procedures required for separating leukocyte-rich plasma from whole blood, and the insensitivity of the method for differentiation of granulocytes from monocytes, limit its practical application.

The Cellscan-Glopr™ mimics human visual identification procedures by computer-TV image processing. It automatically operates a microscope substage to find, frame, and focus leukocytes on the stained blood film. Illuminating with yellow light, it scans the image point by point and quantitates density with a high-speed special-purpose computer and Golay logic processor. It then repeats the process with green light. Each cell is classified by computer comparison with

previously analyzed stereotypes of neutrophils, monocytes, lymphocytes, and other blood cells.<sup>96</sup> The criteria for differentiation are: cell size, cell shape, and the differential absorption of light by the cytoplasm and its granules at the two wavelengths used; the total area of the nucleus and the presence of concavities, lobes, and fine structure within it. The computer has been programmed to separate up to eight cell types.<sup>96,146</sup> Although this system has the potential of most closely duplicating human interpretation of the blood film, its speed, precision, and accuracy remain to be established in extended trials. However, since the theory of cell recognition by computer is well established<sup>173a,173c</sup> and image processing by computers after analog to digital conversion has been used successfully in a number of applications,<sup>3b,173b</sup> including some in hematology,<sup>97a,173a</sup> the prospects for accurate results with this method are good. Furthermore, computer programs have been written for automatically developing the classification algorithms for the computer's decision-making process from an operator's decisions as he classifies cells.<sup>3a</sup> This technique will greatly simplify further applications of this method.

The LARC™ (Leukocyte Automatic Recognition Computer) is an instrument that performs differential leukocyte counts by computerized pattern recognition.<sup>129a</sup> In about one minute the LARC locates 100 leukocytes by a patterned search procedure of a Wright-stained slide of a blood film, and classifies the cells into one of seven groups: band neutrophils, segmented neutrophils, lymphocytes, monocytes, eosinophils, basophils, and the category "others," representing cells that could not be identified as belonging to the foregoing groups. Then, by using the computer's record of the (x,y) coordinates for each of the cells classed as "other," those cells are, in turn, automatically brought into the field of view of the system's binocular microscope for their visual identification by the operator. The computed differential count is automatically adjusted according to the outcome of the operator's classification of those cells. When only normal cell types are

present, definitive classification will fail for about 5% of the cells if the blood films are made on glass slides by using a special centrifugal spreader that the manufacturer claims will produce more even blood films and more uniform distribution of leukocytes, with less cell destruction, than would otherwise be possible.<sup>128a</sup> A preliminary report of the results of a clinical trial with this instrument has been made.<sup>167a</sup> For the screening of routine hospital or laboratory blood films by leukocyte differential counting, the LARC competes favorably against a technologist for accuracy while surpassing the technologist in speed. However, the role of the LARC seems to be to augment the technologist's performance, rather than to replace his evaluation of the blood film, since identification of "other" cells and the description of the morphologic appearance of the erythrocytes and platelets, any one of which may be crucial to a given case, still require human intervention. Greater clinical trial than that presently experienced will be required to determine whether this machine's contribution to the evaluation of the blood will be medically satisfactory and economically advantageous.

The Hemalog-D (trademark of the Technicon Corporation) is a commercially available instrument which automatically determines the leukocyte differential count in a flowing stream of cells at the rate of 60 samples per hour.<sup>154</sup> Whole blood samples of 0.52 ml are diluted, fixed, and then subjected to specific histochemical reactions in each of three simultaneously operating channels. Interfering signals due to erythrocytes are avoided by hemolysis and by the addition of propylene glycol to render the residual stroma translucent. By means of an improved "sheathed" stream control, a single file of leukocytes passes the light beam focused at the flow cell in a system akin to the darkfield light-scattering instrument described earlier. All leukocytes scatter the light forward, through a beam splitter and beyond the darkfield stop disk, to the first photodiode. Cells giving a positive histochemical reaction in a given channel also absorb light, and this is detected in the split beam by the second



photodiode. Pulse amplitude analysis for both light scattering and light absorbance in a given channel provides a two-dimensional vector "description" of each cell which, together with the proper thresholds, is claimed to be unique for each cell type. The three channels for differential histochemical reactions are: (1) peroxidase, at pH 3.8 with 4-chloro-1-naphthol, which stains *eosinophils* deeply and *neutrophils* lightly; (2) lipase, at pH 6.25 in a caccodylate buffer (to block interference from plasma lipase) with  $\alpha$ -naphthyl butyrate as a substrate and hexazonium pararosanilin as the stain for *monocytes*; (3) toluidine blue stain, for *basophils*. In a given channel, within the limits of the upper and lower threshold values, the proportion of cells absorbing light relative to the total number of cells enumerated simultaneously by forward light scattering yields the percentage of the specified cell type in the "differential." Small lymphocytes are determined from the peroxidase channel as the proportion of cells not reacting, hence not absorbing, light (ie, "agranulocytes"); monocytes and platelets are excluded by upper and lower threshold settings, respectively. The sum of the percentage represented by monocytes, small lymphocytes, basophils, eosinophils, and neutrophils, determined by histochemical reaction and pulse-amplitude analysis, is claimed to ordinarily equal, or very nearly equal, 100%. Large lymphocytes, leukoblasts, and other cells which have failed to take the histochemical stains are grouped in the category designated "unclassified cells," with the value derived by subtracting the sum of lymphocytes and monocytes from the "total agranulocyte count" determined in the peroxidase channel.

Thus system uses the combination of histochemical distinctions among leukocytes and cell sizing in a sophisticated optical-electronic system for differential leukocyte counts. The automated system enumerates 10,000 leukocytes in each of three channels for the primary data, whereas the conventional, visual differential counting method classifies only 100 or 200 cells. Whether the large number of cells enumerated will significantly enhance

precision, as has been suggested,<sup>154</sup> not only depends upon the replicability of instrument performance and analysis, but also upon how well this new method for classifying leukocytes compares with the conventional criteria both for patients with nonhematologic disorders and the more challenging patients with abnormal leukocytes. The ultimate usefulness of the instrument which undertakes routine processing of all blood specimens in a large hospital or independent laboratory will depend upon its sensitivity in detecting abnormal cells from among the blood samples from many patients with various diseases. Until favorable performance is established by independent competent trial, conventional procedures for examination of the stained blood film must be followed. Even if suitable accuracy in the automated determination of the differential leukocyte count is proven, the physician must avoid the pitfall of considering this determination as a substitute for the microscopic examination of the blood film. Hemalog D makes no pretense at describing qualitative changes in red cell and platelet morphology, which are important elements of the examination of the blood film. Furthermore, cells classed as "unknowns" by the machine may be of great diagnostic significance and require visual identification and interpretation.

Standardization is essential for even the most precise method, if accurate ("true") results are to be obtained. The impressive reproducibility of results from automated blood-counting machines can lead an observer to the conclusion that these instruments are more accurate than other, less sophisticated, semi-automated, or even manual methods for blood counting, even when the results of the multi-parameter instrument are inaccurate owing to inadequate or infrequent calibration and standardization. Nationwide, interlaboratory quality-control studies have shown serious discrepancies in results with electronic counters, even when standards were sent to the participating hospital laboratories, along with the "unknown" samples.<sup>117</sup>

Excellent standardization of hemoglobinometry<sup>61</sup> is available for the instruments that

measure cyanmethemoglobin. On the other hand, a comparable, stable, absolute reference is not available for particle-counting and cell-volume computation. Pollen grains,<sup>69</sup> latex particles,<sup>155</sup> sodium fluoride-treated yeast cells,<sup>113</sup> preserved blood,<sup>177</sup> and fixed red cells<sup>132</sup> or whole blood<sup>116</sup> have been proposed as intermediate reference standards for comparison in a given laboratory from day to day, and among different laboratories. These still do not represent a primary standard, and validation of reference standards remains a significant, unresolved problem. Present attempts at standardization require comparison with still other instruments or, eventually, the less precise but traditional reference point of manual hemocytometer techniques. In the latter, one should not lose sight of the fact that 17 to 26 replicate counts may be required to reduce the zone of uncertainty about reported results to  $\pm 2\%$  for one standard error. The laboratory which attempts to adjust or standardize an electronic counter with only a single pair of hemocytometer counts, no matter how closely the two agree, or with commercial standards of unproven stability or accuracy, is risking significant error. Carrying forward refrigerated EDTA blood samples from one day to the next serves as a simple but useful quality-control feature for the precision and stability of instruments in the automated laboratory,<sup>27</sup> but does not establish the accuracy of the results.

The physician who interprets results of blood counts must select a laboratory to serve him with knowledge of its accuracy, precision, and quality-control procedures. Unless these qualifications are satisfactory, spurious and variable results may mislead him. A more serious consequence of poor laboratory performance may be the physician's condemnation of established rules and procedures for diagnosis and choice of therapy, when in reality faulty laboratory data were responsible for his erroneous conclusions and actions. Lack of confidence in the morphologic classification of anemia (Chapter 13) often may be traced to reliance upon faulty laboratory data. Throughout his work, the physi-

cian must keep foremost in his mind the understanding that the validity of any information processing procedure, carried out by himself or by a laboratory computer,<sup>23</sup> is wholly dependent upon the validity of the data input. This dependence was recognized in reference to the solution of diagnostic problems in clinical hematology long before it was popularized by modern-day, sophisticated computer operations, with the aphorism: GIGO (garbage in, garbage out).

In summary, electronic cell-counting instruments have the capacity to reduce the error of the blood-counting procedures (Table 1-4) to  $\pm 1\%$  (1 C.V.). However, automation<sup>16,24,33,36,55,56,135</sup> alone, in the absence of strict calibration and quality-control supervision,<sup>58</sup> can produce inaccurate data ad infinitum. The physician must avoid the trap of accepting the output values of automated instruments without the certain knowledge that the correct control procedures are enforced by the laboratory director. This problem has been reviewed in depth.<sup>24,36</sup>

### Examination of Blood by Microscopy

Qualitative evaluation of the morphologic features of the erythrocytes, leukocytes, and platelets by examination of blood films can

**Table 1-4. Accuracy of Blood Counting Procedures**

(Automatic dilutors and electronic cell counters compared with traditional hemocytometer method)

METHOD CELL COUNTED	Two Coefficients of Variation	
	Hemocytometer <sup>1,3</sup>	Electronic <sup>2,4</sup>
Red Cell	$\pm 11\%$	$\pm 3\%$
White cell	$\pm 16\%$	$\pm 4\%$
Platelet	$\pm 22\%$	$\pm 7.6\%^5$

1 Minimal error

2 Usual error

3 Source: Reference 41

4 Source: Author's unpublished data and References 24 and 41

5 The error may be much greater when the platelet count is less than  $35 \times 10^9/l$  or more than  $450 \times 10^9/l$

contribute more toward the diagnosis of conditions which affect the blood, and do so more often, than can any other single laboratory procedure. In addition, parasitemia (malaria, microfilaria) and, rarely, evidences of bacteremia may be found. Three techniques may be used: fixed, polychrome-stained films; wet preparations, examined by phase-contrast microscopy; and wet films, examined by the supra-vital staining method, often in conjunction with phase contrast. Two special methods use electron microscopy: thin section (transmission) and scanning. Electron microscopy is usually reserved for research applications, because of its complexity and expense and the limited occasions upon which it might contribute more to the clinical situation than would standard techniques.

Most widely practiced is the interpretation of the fixed, and stained, *thin blood film*. A valid interpretation depends upon successful completion of three elements: preparation, staining, and examination. The first two are usually the responsibility of technologists rather than physicians. However, they are discussed here because so much depends on their quality and, if problems arise, the hematologist may be severely hampered in his work unless he can resolve them. The techniques are so simple that it is practical, when his work does not warrant the expense of a fully qualified technologist, for the physician to train an office assistant whom he can supervise, rather than forego the opportunity of examining blood films himself in his office. Whether the physician examines the blood films made in his own office, or those made elsewhere, and even when considering reports from technologists and other physicians, it is advantageous to keep in mind the factors which affect the quality of the results.

#### *Preparation of Blood Films*

Films made on coverglasses are preferable, in our opinion, to those made on slides, primarily because a much greater proportion of the blood on the film is technically suitable for examination. Irregular distribu-

tion of leukocytes and platelets occurs on slides,<sup>49,123,131</sup> and it is difficult and sometimes simply not possible to examine the entire slide adequately to determine if the thin zone at the end is representative of the entire specimen and is a reliable basis for interpretation. Sometimes most of the platelets are clustered at the end of the slide where the film is too thick for evaluation, so that the remainder suggests thrombocytopenia. Nevertheless, satisfactory preparations can sometimes be made on slides and some experts prefer them to coverglasses.<sup>49</sup> This is mainly because slides are less difficult to clean than coverglasses and also, being less fragile, they require less care in handling. Laboratories geared toward automation point out that a number of automatic slide-staining machines can work with glass slides<sup>133</sup> whereas only some brands can stain coverglasses.<sup>24,51</sup>

#### *Coverglass Technique*

A good grade of flat, number 1½, square (22 × 22 mm) coverglasses, 0.13 to 0.17 mm thick, are required. They must be free of grease, lint, and dust, and be scrupulously clean; otherwise the blood will not spread and the smear will be totally useless. Rarely, brands are so clean that they may be adequately prepared simply by wiping them with a lint-free cloth or finely woven paper, provided one's hands are first washed free of natural oils and the coverglasses are handled only by their edges. Usually it is necessary to first clean the coverglasses with a detergent and water, then rinse them with hot water, distilled water, and, finally, 95% alcohol. They are then wiped as noted above, and stored in a clean, sealed box. Plastic cover-slips which are "unwetttable" are not suitable.

Details of the procedure are described in earlier editions of this book, and in a manual of laboratory hematology.<sup>41</sup> The chief points to keep in mind are: (1) use a small drop of blood, only 2 to 3 mm in diameter, taken either from a stylet wound, as described earlier (page 6), or from the syringe or needle tip used in venipuncture immediately after the venipuncture has been performed (anti-

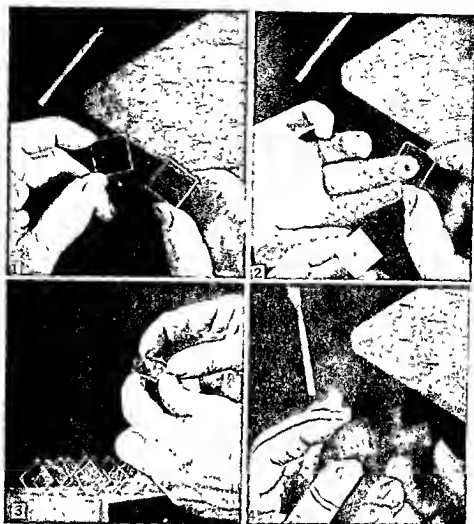


Fig 1-6. The preparation of blood films on coverglasses. Notice how the coverglasses are held in (1). They are crossed in (3) to form an eight pointed figure, and the weight of the upper coverglass spreads the droplet of blood. After about two seconds, opposite edges of the upper and lower coverslips are grasped and they are gently but quickly pulled apart with a motion parallel to their flat surfaces. Care must be taken not to lift them apart. (From Haden, *Clinical Laboratory Methods*, courtesy of C. V. Mosby Co.)

coagulants are not to be used as they will alter the morphologic appearance); (2) hold the coverglasses only by their edges, placing one crosswise over the other, and allow the blood to spread between them for about two seconds; (3) quickly but gently separate the coverglasses by pulling them laterally, in opposite directions to one another but in the plane of the spreading film, just before the film reaches the edges (Fig. 1-6) (do not squeeze or lift the coverglasses from one another); (4) quickly air-dry the films, either by placing them face up on a clean surface if the humidity is low, or by moving them

through the air while holding them by their edges with your fingertips.

If the procedure has been carried out successfully, the blood will be spread evenly and there will be neither holes nor thick areas in the preparation. A multicolored sheen will be seen on the surface of the dried, unstained film if light glances off from it at the proper angle, for the thin layer of closely fitting cells acts like a diffraction grating. Later, under the microscope, after staining, the red cells will be seen next to each other, but neither overlapping, nor in rouleau formation, and central pallor will be visible; lymphocytes

will have a readily distinguished cytoplasm, rather than a minimal zone bearing closely on the nucleus as occurs in thick films or those which dry too slowly.

### Slide Technique

The slides must be clean, as noted above, but often not as painstaking a technique is followed as that used for coverslips. Certain brands are suitable as cleaned by the manufacturer, when delivered in cellophane-wrapped and sealed boxes. A somewhat larger drop of blood than that used on cover-glasses is placed on one end of the surface of a slide. It is spread by means of another slide held at about a 30-degree angle to the first slide, and placed at first slightly in front of the drop and then moved back until the drop spreads out at the interface. The spreader is then pushed forward at an even, moderately rapid rate. Faster movement produces thicker but more even films. Centrifugal devices which make blood films are now available. A device has been manufactured to concentrate cells from a suspension onto a microscope slide.<sup>172</sup> The criteria for satisfactory films are the same as noted above. Drying is accomplished as with cover-glasses or, taking advantage of their greater weight, the slides may be placed on a surface and fanned. Fixation occurs during the first minute of staining if the stain, eg, Wright's, contains absolute alcohol.

### Staining

Several modifications of the Romanowsky method of staining are used. The most popular stains are Wright's and the May-Grünwald-Giemsa. These stains for blood smears have as their base a "polychromed" methylene blue, which is metachromatic.<sup>118</sup> Alkalization and subsequent addition of eosin (Malachowski 1891) have replaced the original method which required old, moldy methylene blue. In Giemsa's stain, "methylene blue azure" is added to a mixture of methylene blue and eosin.<sup>164</sup> Details of the staining technique are given in earlier edi-

tions of this book and elsewhere.<sup>41</sup> A properly stained film will appear pink to the unaided eye. Microscopically, the erythrocytes will appear to be pinkish-orange in color, the nuclei of the leukocytes purplish-blue, and neutrophil cytoplasm tannish with violet-pink or lilac granules, while eosinophil granules will be a bright orange-red. When Wright's stain is used, if the whole film is *too blue* and the red cells are stained blue or green, the trouble may be due to (1) too prolonged a staining time, (2) too alkaline a buffer, (3) too little washing after staining, or (4) too thick a smear. To improve the color, less stain or more diluent may be used, or the length of time employed for staining may be decreased and that for washing increased. To counteract the effect of an excess of alkali in the stain, some acetic acid may be added. Sometimes the stain is improved by diluting it with an equal part of absolute methanol. If the stain is *too red*, the nuclei of the leukocytes are pale blue, erythrocytes are bright red, and eosinophil granules stain a brilliant red-orange. This may be due to (1) excessive acidity of the stain or the buffer, (2) too short a staining time, or (3) excessive washing. The buffer must be controlled between pH 6.4 and 6.7. The choice is made according to the color balance desired on the smear. For best results, staining and buffer times must be established for each batch of stain. The concentration of the dyes in Wright's stain is continually changing, owing to progressive oxidizing action in the alkaline solution. Other problems are the loss of methanol from the stain due to evaporation and the acquisition of water vapor by the hygroscopic absolute methanol in the stain. For these reasons, bottles of stain must be kept tightly closed. Artifacts which simulate hypochromic red cells may be produced by water in the Wright's stain.<sup>14</sup> After the buffer has been on the smear for the required time, if one is to avoid leaving precipitates on the surface of the film, the coverglasses must be flooded with water rather than tilted to rid them of stain. Precipitated stain obscures details, may be confused with cocciform bacteria, and can simulate red cell inclusions.

## Examination

The microscopic examination of the blood film can provide more useful and important information for the evaluation of hematologic problems than can any other single test. The blood smear should be examined for more than the differential leukocyte count. Astute deductions regarding the concentration of leukocytes and platelets in the blood can be made, thus guarding against major faults in the reported counts; also the red cell and platelet morphology may be assessed and a search can be made for foreign elements. A systematic approach is important.

First, the blood film should be examined, at about 100X magnification (low power), for its adequacy of cell distribution and staining and for freedom from artifacts. We make it a practice to examine at least two coverglass films from any single specimen. The important features of cell distribution are illustrated in Figure 1-7. Signs of a poor film are: disruption of leukocytes, overlapping or aggregated red cells, or areas almost entirely comprised of cells with (1) uniform loss of central pallor, (2) polygonal shapes—excepting characteristic poikilocytes (eg, sickle cells, helmet cells), and (3) "spherocytic" appearance. True spherocytes, in contrast to artifacts, usually show some variation in central pallor and are usually smaller than normal cells, whereas artifacts are larger.

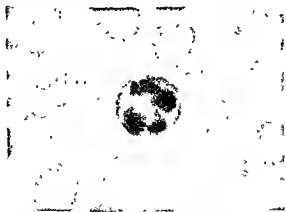


Fig 1-7. The arrangement of red and white blood cells in a correctly made blood film for microscopic examination

If the preparation is not satisfactory, one should not proceed further. Other stained films should be examined, or new preparations should be made. Quite misleading impressions can be gained from attempts at interpretation of substandard blood films. This fact is not fully appreciated by technologists and physicians who deal mainly with routine blood examinations, for, by chance alone in view of their material, their error ratio will be low. Nevertheless, an error of omission, or an incorrect interpretation, places an additional burden on the patient and his physician. From the patient's point of view, an error which could have been readily avoided is not easily forgiven. In laboratories with a heavy work load, a conflict of interest is created for the technologist who is concerned with the adequacy of a given preparation and who is considering devoting additional time to making a new preparation. It is up to the physician in charge of the laboratory to see that quality is not sacrificed for speed. It is a good practice to save blood films, even in routine laboratories where they may be kept for at least two weeks, to allow an opportunity for further evaluation in retrospect and to serve as a quality control measure in the event surprising or conflicting findings appear later. In patients with hematologic problems, it is essential that the blood film be examined by a qualified physician or his consultant.

If the blood films are satisfactory, the examiner should estimate the leukocyte count by surveying many fields, keeping in mind the normal situation. The findings should be compared with the reported count when it becomes available.

Next, under about 1000X magnification (switching to the high-power, oil-immersion lens), the red cells are examined for abnormalities in size (macrocytosis, microcytosis) and the degree, if any, of variation in size (anisocytosis) or shape (poikilocytosis), or reduction in the depth of the hemoglobin color (hypochromia), and for variation in color (polychromasia). One must also look for basophilic stippling and the presence of inclusions (eg, nuclei, Howell-Jolly bodies,

Cabot rings, and parasites). The significance of these findings is discussed in Chapter 13.

Next, under 1000X magnification, the numbers of platelets are estimated in several successive fields and compared with their frequency in blood films from normal individuals. One must be certain, from the examination under low power, that clumps of platelets are not present in a few areas, for, if this is the case, a fair appraisal of the platelet count cannot be made by examination of random fields as described. The size (normally, 2 to 5  $\mu$ m) and shape of the platelets are also recorded. The significance of these findings is discussed in Chapters 9, 36, and 37.

After the *leukocyte* count has been estimated by surveying the film under the 100X (low-power) magnification, the differential count is made under about 1000X magnification (Chapter 6). The significance of abnormalities in the leukocytes is discussed in Chapters 42 and 48 to 50.

One must be alert for clues which have bearing on other organ systems, or other aspects of the blood. For example, hypersegmentation of neutrophils may be a clue to anemia caused by deficiency of vitamin B<sub>12</sub> or folic acid (Chapters 6 and 14). Basophilic stippling of red cells may be a clue to neuropathy due to lead poisoning (Chapter 19) or to thalassemia minor (Chapter 27). The obscure cause for severe fever may be solved by the discovery of a microgametocyte in a red cell (Plate XI). Such observations require more care and discernment than does the recognition of the marked lymphocytosis which characterizes chronic lymphocytic leukemia, but they are no less significant to the alert physician and to the patient who depends upon the physician for health care.

### Special Histochemical Stains

The differentiation allowed by any staining reaction is simply a reflection of the differences in chemical composition, reactive acidic or basic groups, and the like, in adjoining structures. The irregular distribution of certain enzymes within any given cell, or among cells of different types, is also used to advan-

tage.<sup>150</sup> The details of these more complex procedures are to be found elsewhere. The principles and main findings are reviewed here. In each case, a "positive" reaction is indicated by the development of a particular color, or the precipitation of granules or other matter specific for the reaction. These localize the substance in question.

### Peroxidase Reaction

This detects an enzyme present in promyelocytes and later cells of the same series and to a lesser degree in monocytes (Plate XX). It is most useful in classifying acute leukemia (Chapter 47), when it may reveal promyelocytes which have too few granules to be visible for certain identification in a smear stained with Wright's reagent. A positive reaction is indicated by blue granules or crystals in the cytoplasm. Lymphocytes and lymphoblasts give a negative peroxidase reaction; myeloblasts have been said to give a negative reaction, but it is a matter of semantics to decide whether leukoblasts which appear to be devoid of granules but which nevertheless give a positive peroxidase reaction should be termed myeloblasts or promyelocytes (Chapter 6).

### Leukocyte Alkaline Phosphatase

This can be quantitated by biochemical procedures on separated, lysed leukocytes (Chapter 6); however, valuable semiquantitative information is obtained by a staining procedure. The enzyme hydrolyzes the substrate naphthol AS-BI phosphate to an aryl naphtholamide which is, in turn, coupled to a soluble diazonium salt, forming an insoluble red azo dye which precipitates at the location of the enzyme.<sup>99</sup> Alkaline phosphatase is present in neutrophilic leukocytes. Increased amounts of activity are found in neutrophilia accompanying infection and in a wide variety of other conditions (page 1279), and in women taking estrogen-progesterone combinations for contraception. Low activity is found in chronic myelocytic leukemia,<sup>102</sup> paroxysmal nocturnal hemoglobinuria,<sup>83</sup> and hypophosphatasia.<sup>4</sup>

### Periodic Acid-Schiff (PAS) Reaction

This detects intracellular glycogen. Megakaryocytes and platelets are richly positive, granulocytes range from a negative reaction in myeloblasts to strongly positive in segmented neutrophils,<sup>90</sup> monocytes often show a few granules, and lymphocytes occasionally show a slight positive reaction. Normal erythroblasts and mature erythrocytes are entirely negative, except in patients with thalassemia (Chapter 26) and those with erythremic myelosis<sup>148</sup> (Chapter 48).

### Sudan Black B

This stain detects lipids. By the improved method of Sheehan and Storey,<sup>158</sup> granules in leukocytes are stained. Leukemic myeloblasts may give a strongly positive reaction, although this is not uniformly so. The reaction is of greatest value in identifying as myeloblasts those cells of a patient with acute leukemia which otherwise appear undifferentiated when examined by Romanowsky stains and which are negative with the peroxidase reaction.<sup>91</sup> Auer rods are strongly sudanophilic.<sup>91</sup> Lymphoblasts, erythroblasts, and lymphocytes give a negative reaction. A positive reaction is found in myelocytes, metamyelocytes, and polymorphonuclear leukocytes of both the eosinophilic and neutrophilic series, most prominently so in the latter.<sup>91,149</sup> Monocytes show minute positive granules, while granules of basophils are variably positive.

### Other Stains

A modification of the Feulgen reaction for deoxyribonucleoprotein is useful in demonstrating nucleoli.<sup>178</sup> The latter will appear as unstained spaces. A methyl-green-pyronin stain affords some measure of the ribonucleoprotein content of leukocytes.<sup>143</sup>

The reticulocyte stain, new methylene blue N, precipitates erythrocyte ribosomes into a network which is visible by light microscopy (Chapter 3).<sup>19,98</sup> It is a most valuable tool for appraising the rate of effective erythropoiesis (Chapters 13 and 20).

The Prussian blue reaction utilizes a fresh, acid solution of potassium ferrocyanide. It detects non-heme iron, ie, *ferritin* and *hemosiderin* iron, in red cells (siderocytes, Chapters 3 and 4), marrow (Chapter 2), and in urine where its detection is of significance in patients with hemolytic transfusion reactions (Chapter 27) or in those with intravascular hemolysis, such as paroxysmal nocturnal hemoglobinuria (Chapter 29) or hemochromatosis (Chapter 4). The same reagent is used to detect hepatic iron excess in hemochromatosis.

Heinz bodies are denatured hemoglobin and have the same tinctorial characteristics as the native protein. They are not visible with Wright's stain. *Crystal violet* will reveal them in a wet preparation as deep purple, irregularly shaped small bodies in the red cells. They may be the result of oxidant drugs (Chapter 23) or unstable hemoglobins (Chapter 24) and may be seen in splenectomized individuals, particularly those with thalassemia (Chapter 26). *Brilliant cresyl blue* will stain reticulum and inclusion bodies, and will precipitate unstable hemoglobins such as Hb Zurich and Hb H.

### Examination of the Wet Film of Blood

Wet preparations are valuable for the demonstration of parasites in blood, some organisms being more easily detected by their pigment (malaria) or by the currents and disturbance they produce in wet films (spirochetes, trypanosomes) than after they have been stained. Wet preparations may be used with or without the addition of sodium metabisulfite for the demonstration of sickling of the red corpuscles (Chapter 25). In fresh, wet preparations the motility of the leukocytes is preserved and their activity and phagocytic power may be studied. Supravital staining is a modification of this technique, dye being added for the purpose of staining the mitochondria and vacuoles (see below). Rouleau formation may be observed; and, after the specimen has stood for 10 or 15 minutes, increase or decrease of fibrin may be roughly gauged.



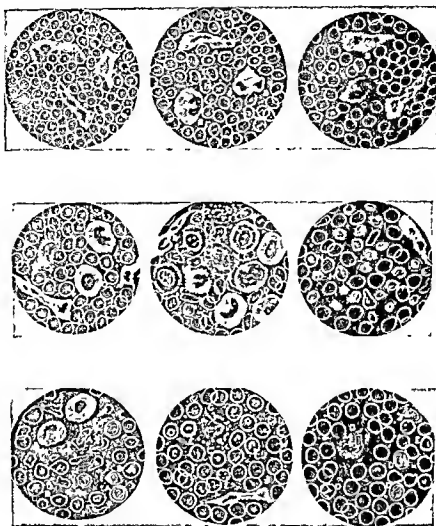


Fig 1-8. Camera lucida drawings of fresh (human) blood preparations as seen by darkfield illumination. In 1, 2, and 3, adult myeloid granulocytes are shown in various phases of maturation. A, neutrophilic leukocyte; B, eosinophil; C, basophil. In 4, 5, and 6, A and B are myeloblasts; C, D, early myelocytes; E, neutrophilic myelocyte; F, eosinophilic myelocyte; G, basophilic myelocyte; H, lymphoblast; I, J, lymphocytes. K, monocyte; N, normoblast. No. 7 is from a patient having erythroblastic anemia. 8 and 9 from patients infected with *Plasmodium vivax*. In 7, 8, and 9, A is a nucleated red cell; B, erythrocyte containing nuclear chromatin; C, young trophozoites; D, ameboid trophozoites; E, crenated erythrocytes; F, lymphocytes; G, monocyte. Magnification approximately X800. (From Hansen-Pruss<sup>91</sup> courtesy of author and Williams & Wilkins Co.)

A drop of blood from the finger or earlobe is placed on a coverglass. The coverglass is inverted on a glass slide and sealed to the slide with petrolatum or paraffin. The drop of blood should be only slightly larger than a pinhead. If the glassware is clean and the coverglass is deposited gently on the slide,

the blood will spread out evenly under the weight of the coverglass. Pressure should not be applied. The specimen is first examined with the low-power lens of the microscope in order to determine whether it is satisfactory, and then the oil-immersion lens is used. The red corpuscles should lie slightly

separated from one another. Their size, shape, and color may be noted in addition to the other features already discussed. If the microscope condenser is lowered slightly, contrast may be improved and the examination made more easily.

### Darkfield Illumination

Darkfield illumination facilitates the examination of the wet blood film<sup>80</sup> (Fig. 1-8). The leukocytes are exceptionally well seen by this method, for their granules are refractile and set off the nucleus in sharp contrast. Erythrocytes are readily made out because the pericellular membrane is highly refractile. Normoblasts may be distinguished by a faintly perceptible perinuclear membrane. Unstained reticulocytes cannot be distinguished, but, after staining with new methylene blue N or brilliant cresyl blue, the reticulum stands out strikingly. Blood platelets are easily seen because of their refractility and, in addition, numerous small round or dumbbell-shaped particles ("blood dust," hemokonia of Muller) which manifest active brownian movements are found. These may be granules of disrupted leukocytes. Fibrin crystals appear as fine, slightly refractile needles. Malaria plasmodia stand out with clarity, the pigment being highly refractile. Since the parasites remain viable for 10 to 16 hours in these preparations, their growth and development may be observed.

### Supravital Staining<sup>97,114,156,163</sup>

Supravital staining, introduced by Cowdry, was developed and popularized by Simpson and, particularly, by Sabin<sup>153</sup> and her students.

The advantages of supravital staining are: (1) The appearance of the *living, motile cell* is revealed. (2) The method *avoids cell damage* when blood or bone marrow preparations are made.<sup>151</sup> The morphologic types may be readily identified<sup>1,156</sup> and differential counts may be performed reliably. This avoids the problems of interpretation which are espe-

cially prone to occur in leukemia when, in fixed, thin bone marrow smears made in the regeneration phase after chemotherapy or radiotherapy, more than a minor number of cells are damaged in making the smears and cannot be identified after staining.<sup>151</sup> (3) Red cell *inclusions* may be readily seen if appropriate dyes are used; eg, methyl violet reveals Heinz bodies (*vide supra*). (4) *Cell density* and architecture of the aspirated bone marrow spicule are displayed in more nearly their natural state than in thinly spread, pulled, and fixed marrow smears and films.

The disadvantages are: (1) The preparations are not permanent. (2) They must be examined when fresh. (3) They are not suitable for radioautography.

The technique of Forkner,<sup>65</sup> or derivations of it,<sup>38,49</sup> is used. A mixture of neutral red and Janus green in absolute ethanol is flooded onto the surface of a chemically clean slide. Excess dye is drained away and the remaining film on the slide is quickly evaporated by applying heat. In this way a thin, even film of dye is dried onto the slide. The stained side of the slide is marked at once, for it is difficult to identify later.

Pinacyanol (Eastman Kodak Organic Chemicals, Catalog No. 622) has advantages over Janus green as the mitochondrial stain.<sup>156</sup> With rare exceptions, Pinacyanol does not fade out of mitochondria for at least 24 hours, and it stains the nuclear chromatin a purplish blue without interfering with cell viability or motility. It does not impede the staining of vacuoles by neutral red. At a concentration which stains nuclei, Janus green kills the cells. One ml each of 0.4% neutral red and 0.03% Pinacyanol is added to 5.0 ml of absolute ethanol. The freshly prepared mixture is used to make the slides, as above. Since Pinacyanol is light sensitive, the solutions and the slides should be kept in a dark, cool place.

A drop of blood is gently placed on the marked surface of the prepared slide. The preparation is rimmed with petrolatum. The blood may be examined within 15 to 30 minutes; or, if delay is necessary, the preparation may be stored in the refrigerator at a

temperature of about 4° C for as long as 24 hours. A microscope stage, warmed to 37° C, facilitates movement of the leukocytes during study of the blood, but a warmed stage is not essential for identification of the cells.

For successful supravital staining it is necessary (1) to have clean glassware and (2) to avoid the use of so great a concentration of dye that the cells are killed. Death of a cell is indicated by staining of the nucleus with Janus green. A greater quantity of dye must be used for abnormal bloods with a greater than normal number of leukocytes than for normal specimens. In a good preparation the Janus green or Pinacyanol stains the mitochondria a brilliant blue-green, while the neutral red stains the specific granules and vacuoles of the leukocytes in varying shades, depending on their pH: basophilic granules are brick red, neutrophilic granules faint pink, and eosinophilic granules bright yellow. (For detailed descriptions of leukocytes stained "supravitaly," see Chapter 6.)

For the study of bone marrow, lymph nodes, or other tissues where there is an abundance of colorless cells to be stained, more dye is required than for the examination of blood. Imprints may be prepared from a freshly cut, moist surface of tissue. The prepared slide is firmly pressed against the tissue surface for a few moments, without applying any shearing force. Cells will adhere to the slide. If a thin film of intact tissue cannot be obtained, the cut surface is scraped with a sharp scalpel and the tissue accumulating on the knife blade is placed on the slide prepared with the dyes.

### Phase Contrast

Differences of optical path are translated by the phase-contrast microscope into light and shade.<sup>104, 119</sup> Particles which differ in refractive index from the medium in which they are immersed consequently appear brighter or darker than their surroundings. This technique offers greater detail in the study of the finer structure of living, unstained and unaltered cells than is otherwise available. Thus chromatin, mitochondria, the centrosome, and the specific

granules of the cells can be seen clearly, often more distinctly than in stained preparations.<sup>8,9,11,131</sup> Howell-Jolly bodies can be recognized as bright, round bodies inside the erythrocyte; Heinz-Ehrlich bodies as distinct, dark bodies. The various forms of granulocytes can be differentiated by the different appearance of their granulation. Platelets are seen so well that this is the preferred means for their enumeration by hemocytometer technique.<sup>20</sup> The cells of infectious mononucleosis manifest a black and coarse granulation in their cytoplasm which differentiates them from monocytes and ordinary lymphocytes. In certain types of reticulum cells, and especially in myeloma cells, dark drop-shaped formations which have not been found in other cells have been observed adjacent to the nuclear membrane. Distinguishing features have also been described in leukemic and lymphosarcoma cells, lymphoblasts, and myeloblasts,<sup>131</sup> as well as in other types of tumor cells. Auer bodies and nucleoli are particularly well seen.<sup>11</sup> Excellent photomicrographs have been published<sup>1</sup> which convincingly establish the fact that phase contrast is far superior to ordinary bright-field microscopy for the examination of the wet, living blood film.

When microcinematography is combined with phase microscopy, cellular activities which have passed unobserved by ordinary techniques can be seen (Chapter 6, page 224).

### Electron Microscopy

Electron microscopy has made possible the study of cellular detail at the level of 1 nm<sup>12</sup> and has provided extremely valuable information concerning the structure of the cells of the blood<sup>10,73</sup> as well as their function.<sup>11</sup> (See Chapters 3, 6, and 9.) It requires special conditions of fixation, dehydration, embedding, and sectioning.

### Transmission Electron Microscopy (TEM)

A beam of electrons directed in an evacuated chamber through an ultra-thin section of tissue supported on a thin grid is focused by



Fig. 1-9. Ferritin in the cytoplasm of an erythroblast, viewed by transmission electron microscopy. The iron is electron-dense and is seen to be arranged at the corners of a regular octahedron. Insets show two granules displaying a characteristic tetrad structure on thin section. (From Bessis and Breton-Gorius,<sup>13a</sup> courtesy of authors and Henry M. Stratton, Inc.)

electromagnets onto a high-resolution photographic plate. Magnifications up to 100,000X and resolution of point-to-point distances of less than 0.4 nm have been achieved.<sup>87</sup> This is about one thousand times better than resolution with the light microscope. The limita-

tion of resolution for TEM, based upon the wavelength of the electromagnetic radiation, has been calculated to be 0.22 nm.<sup>157</sup> The contrast needed for production of an interpretable image is a function of the ability of the specimen to absorb electrons ("electron

density") and of the atomic number. The greater the latter, the greater the capacity of the atomic nucleus to scatter electrons. As a result, TEM reveals atomic rather than molecular properties of the specimen. For example, it is the iron in the ferritin molecule which enables the identification of the ferritin (Fig. 1-9). Stains which interact differently with different molecules in a specimen to produce contrasting colors for light microscopy are not applicable in electron microscopy. So-called "stains" used in TEM actually deposit atoms of high atomic weight, such as lead, osmium, or uranium, sometimes differentially in reacting with tissues. "Shadowing," by overlaying onto specimens in vacuo, from a point source, a very thin film of platinum, gold, or other heavy metals, yields replicas with imposed contrast which can reveal topological features. When combined with freeze-etching<sup>101</sup> or freeze-cleaving, minute structural details of the erythrocyte membrane<sup>171</sup> and of organelles have been depicted (Fig. 3-11, page 94). Although great detail has been revealed, TEM is limited, as is the high-resolution light microscope, to the two-dimensional image (Fig. 3-3 page 85). Furthermore, TEM requires extremely thin sections. Both factors make it very difficult to gain an impression of the three-dimensional structure of the specimen.

### Scanning Electron Microscopy (SEM)

This provides an image of the surface with perspective which imitates the normal stereoscopic vision, but at the higher magnification and resolution allowed by the electron beam rather than the relatively low resolution and much lower magnification imposed by the longer wavelengths of the visible-light stereoscopic microscope. The SEM moves a beam of electrons as small as 5 nm in diameter across the specimen. The interaction of the beam with the specimen produces secondary irradiation which is generally not focused. Specialized detectors for the several kinds of secondary irradiation provide a signal to

modulate another electron beam which is synchronized with the electron beam in the microscope column and which scans across a standard cathode-ray tube (CRT). The modulation regulates the brightness of the CRT image from point to point and the synchronization of the two beams results in information transfer much as occurs in a television picture tube.<sup>88,89</sup> The magnification is the ratio of the size of the scan in the CRT to that in the microscope column as the electron beam crosses the specimen.

In one mode of operation called cathodoluminescence, visible light is produced in the specimen as the electron beam excites naturally occurring substances in biologic materials or in fluorescent stains or other materials (such as fluorescent antibodies) which have been specifically applied to the specimen. In such arrangements, the detector is a photomultiplier tube and the resultant image localizes the fluorescent materials. The great depth of field and resolution of SEM make this a valuable extension of fluorescence microscopy, at 10,000X magnification!

When the secondary irradiation is in the form of "secondary electrons" rather than visible light, it leaves the surface of the specimen with an intensity which depends upon the angle of incidence of the scanning electron beam. Surfaces of the specimen which are oblique or tangential to the electron beam yield a darker image on the CRT than do those at right angles to the beam. The resulting image creates the illusion of three-dimensional viewing, much as does shading in a skillfully executed drawing or a photograph of an obliquely illuminated object. Extraordinary views of blood cells<sup>13,11,129</sup> (Fig. 3-8, page 92) and bone marrow elements<sup>168</sup> have been obtained with this technique (Fig. 1-10). It conveys a different type of information than does TEM and within a few years since its introduction has become a widely used biomedical research tool.<sup>88,89</sup> The x-ray contrast mode of operation of SEM analyzes the spectrum of x rays produced when the electron beam strikes the target. This provides data on the elemental distribution in the specimen.<sup>88</sup>

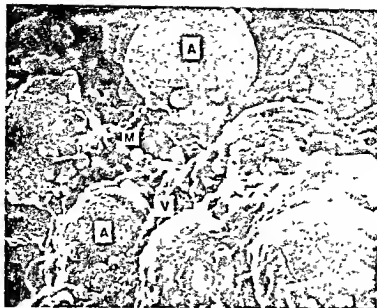


Fig 1-10 Normal human sternal bone marrow seen by scanning electron microscopy at an original magnification of 500X. The aspirated specimen was fixed in 6.5% glutaraldehyde and then 1% buffered osmic acid. The adipose cells (A) are in a loose aggregate and are much larger than the normal marrow elements (M) which appear as globules in between them. V denotes a vessel (From Trubowitz et al.<sup>143</sup> courtesy of authors and Henry M. Stratton, Inc.)

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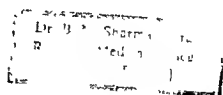
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# Part II

## The Normal Hematopoietic System

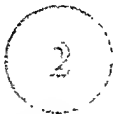


## SECTION 1: *Basic Cytology*

Cells of the blood (erythrocytes, platelets, neutrophils, lymphocytes, monocytes, eosinophils, and basophils) are constantly lost and thus to maintain homeostasis each system must have the capacity for self-renewal. As in all mammalian cell renewal systems, proliferation occurs through cell division. Blood cells other than the lymphocyte and possibly the monocyte differentiate to a point at which cell division cannot or does not occur. Thus, in these systems, renewal involves division of immature cells coupled with differentiation (maturation).

Self-renewal systems must contain *stem cells*. A stem cell can generally be defined as one in which the progeny (daughter cells) of cell division are identical in appearance and potential to the mother cell. A stem cell system which must renew mature nondividing compartments must also be capable of differentiation. Thus, the required characteristics of an hematopoietic stem cell are self-renewal and differentiation.

The production of hematopoietic cells in normal adults is limited to the bone marrow and the widespread lymphatic system. In this section the general principles of cell proliferation and differentiation, stem cell systems, and development of the marrow will be reviewed. The development of the lymphatic system is discussed in Chapter 7.



## *Origin and Development of the Blood and Blood-Forming Tissues*

### **Proliferation and Differentiation of Cells**

#### **Proliferation**

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Mitosis

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Number of Hematopoietic Cells in Bone Marrow

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Methods for Obtaining Bone Marrow Specimens

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Estimation of Cellularity

Cells of the Normal Bone Marrow

Indications for Marrow Aspiration

Indications for Marrow Biopsy

## Proliferation and Differentiation of Cells

Cellular proliferation and differentiation may occur simultaneously in the same cell or may be independent processes.

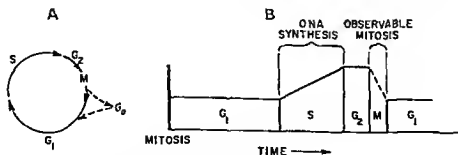
### Proliferation

Cells capable of proliferation may be in a generative cycle (G) or in a resting state ( $G_0$ ) from which they can be triggered into G. During the generative cycle the total amount of deoxyribonucleic acid (DNA) doubles and there is an increase in other cellular constituents such as ribonucleic acid (RNA) and proteins. An equal distribution of cellular constituents to each daughter cell occurs during the process of mitosis. Synthesis of DNA appears to be the key to the generative cycle.<sup>13</sup> Synthesis of RNA and other proteins may proceed during  $G_0$  or in cells incapable of proliferation. Synthesis of DNA is limited to cells in G except for unusual circumstances in which the phenomenon of DNA repair occurs, as in cells damaged by such factors as ultraviolet light<sup>16,17</sup> or x-irradiation.<sup>7,18</sup>

### The Generative Cycle

The generative cycle is divided into four phases which may be represented in a circular fashion (Fig. 2-1A) or as a curve (Fig. 2-1B). In the first, or  $G_1$  phase, RNA and protein synthesis begin and at some point in this phase the process triggering DNA synthesis, as yet undefined, is initiated.<sup>15</sup> In the second phase (S), DNA synthesis occurs and is followed by  $G_2$  and in turn by mitosis (M). Protein synthesis and cell volume expansion<sup>19,20</sup> continue throughout the cycle. In general, once DNA synthesis has begun, the generative cycle must be completed or the cell will die. This phenomenon forms the basis for the cell-killing effects of chemicals which inhibit or block synthesis of DNA or block mitosis (Chapter 55).

It appears that there is a real difference between a very long  $G_1$  state and true  $G_0$ . For example, spermatogonial stem cells appear to exist in a state of true  $G_0$ <sup>21</sup> while epithelial cells of the hamster cheek pouch are thought to be in a very long  $G_1$  state.<sup>22</sup> As applied to the treatment of acute leukemia, this distinction is important because a cell in a long  $G_1$  will eventually enter DNA synthesis and be subject to killing by drugs acting



$G_1$  - NUCLEUS CONTAINS DIPLOID DNA

S - PERIOD OF DNA REPLICATION

$G_2$  - NUCLEUS CONTAINS TETRAPLOID DNA

M - PERIOD OF MITOSIS

$G_0$  - RESTING STATE. HAS POTENTIAL FOR DIVISION

GENERATION TIME - TIME FROM ONE MITOSIS TO THE NEXT

Fig. 2-1 the generative cycle (G)

in that phase. On the other hand, a leukemic cell in  $G_0$  would never become sensitive to such drugs unless induced to leave that state.

Deoxyribonucleic acid is the repository of genetic information which is coded in the long polymeric molecule by variation in the sequence of purine and pyrimidine bases. The four bases of normal DNA are two purines, adenine and guanine, and two pyrimidines, thymine and cytosine. A base-deoxyribose-phosphodiester linkage is present; the phosphodiester links the 5' carbon of one deoxyribose unit with the 3' carbon of the next sugar residue.

The synthesis of DNA can be interrupted by a variety of chemical agents (Chapter 55) and the onset of DNA synthesis is selectively blocked by actinomycin D.<sup>15,203</sup> Since actinomycin D is thought to block synthesis of messenger RNA, the trigger for onset of DNA synthesis may be mediated by messenger RNA. However, the effect of actinomycin D in inhibiting DNA synthesis is not noted for more than an hour after drug administration.<sup>15</sup> Consequently, the DNA synthesis trigger may be reasonably remote in the  $G_1$  phase. Inhibiting protein synthesis with drugs such as puromycin delays but does not inhibit the onset of DNA synthesis.<sup>203</sup>

The post-DNA synthesis, premitotic phase ( $G_2$ ) has been well studied with respect to agents which influence subsequent mitosis (see <sup>15</sup>). The rate of protein synthesis decreases during  $G_2$ . Early in  $G_2$  the inhibition of RNA synthesis by actinomycin D leads to failure to enter mitosis, as does protein synthesis inhibition by puromycin in early or mid- $G_2$ . Puromycin administration in the later stages of  $G_2$  allows mitosis to begin but it is not completed. Thus, it would appear that  $G_2$  is a period of preparation for mitosis which includes key RNA and protein synthesis activities. The time of onset of mitosis appears to be more closely related to cell volume than it does to cell age,<sup>17</sup> suggesting that the amount of material synthesized may trigger mitosis.

The duration of  $G_1$  is quite variable and the primary mechanism for shortening the generation time in order to accelerate the rate

of cell production is by shortening  $G_1$ .<sup>34</sup> In rapidly growing mammalian cell systems the duration of S,  $G_2$ , and M is shortened less than  $G_1$ , if shortened at all.<sup>38,195</sup> In plant cells, on the other hand, it is the duration of S which appears to be the primary variable.<sup>213</sup>

Doubling of DNA content during proliferation reflects the reduplication of each of the chromosomes of man. This reduplication is accomplished under the influence of DNA polymerase enzymes by the production of an exact copy in each chromosome of the sequences of guanine, adenine, cytosine, and thymine of each double helix strand of DNA.<sup>15</sup> Except for cells infected with certain RNA viruses<sup>11,202</sup> and in embryonic or leukemic cells<sup>82</sup> or cells in culture, in which RNA-dependent DNA polymerases are found, the DNA polymerase also is DNA-directed. The overall rate of DNA synthesis may be relatively constant throughout the S phase of the generative cycle<sup>226</sup>; however, different chromosomes predictably begin DNA synthesis before others in human marrow cells<sup>197</sup> as well as in other mammalian cell systems.<sup>99</sup> This suggests that an exact regulatory mechanism of DNA synthesis governs the entire S phase.

### Mitosis

The onset of mitosis is signified morphologically by dissolution of the nuclear membrane and organization of the chromatin into individual chromosomes (*prophase*, Fig. 2-2). Chromosomes with their templated duplicates still joined at the reduplicated chromosomal centromere are then aligned (*metaphase*). The tubular mitotic spindles connecting the chromosomal centromere to the cellular centriole contract and one half of each of the duplicated chromosomes is drawn toward each of the centrioles (*anaphase*). A nuclear membrane is formed around each of the two sets of chromosomes, the cytoplasmic membrane invaginates around the nuclei, and the cell separates (*telophase*). In certain normal cells, such as megakaryocytes, and in a variety of diseases, telophase is not completed

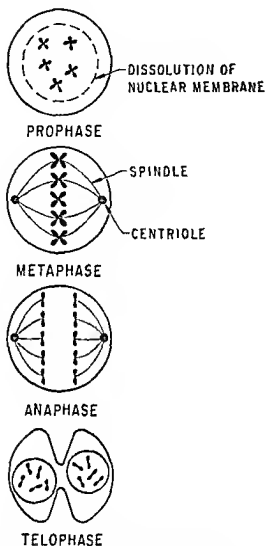


Fig. 2-2 The four stages of mitosis. For simplicity of illustration five rather than the normal 46 chromosomes of man are shown in the diagram.

and multinucleated cells are formed. Such cells are said to be polyploid, but differ from cells with an abnormally large number of chromosomes contained within a single nucleus (Chapter 46).

### Chromosome Defects

During metaphase, if cells are caused to swell and break, chromosome spreads can be prepared and stained (Fig. 2-3A). From these the number of chromosomes can be counted

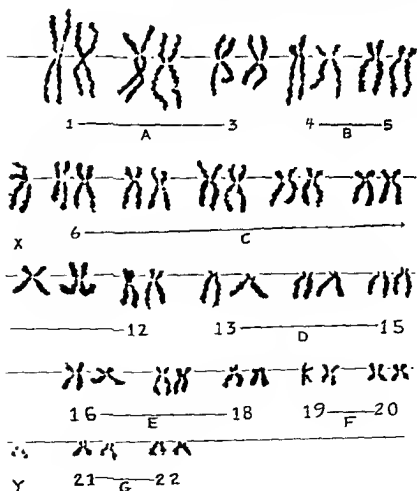
and each chromosome or group of morphologically similar chromosomes can be examined.<sup>117,150,185</sup> The normal number of human chromosomes has been shown to be 46.<sup>72,205</sup> The terminology used in referring to the different normal chromosomes and chromosome groups is presented in Figure 2-3B. Chromosome preparations can be made from any dividing cell system, but are commonly prepared from buccal mucosa or skin cells, blood lymphocytes (induced to divide in culture by the addition of phytohemagglutinin), or bone marrow cells. It is through such examination that chromosome defects associated with a variety of human diseases have been detected.

If a chromosome defect is transmitted by sperm or ovum, or if the defect arises before the first mitotic division of the fertilized ovum, all cells in the body will bear the defect. Chromosome constitution and DNA content generally are constant for all potentially dividing cells in an individual. However, in mature neutrophils a modest loss of DNA may occur.<sup>89</sup> If chromosomes are unevenly divided at the first mitotic division (*abnormal segregation* or *nondysjunction*), theoretically two different defects will be detectable and two different cell lines, one with an extra chromosome and one with a missing chromosome will be present. This circumstance is referred to as *mosaicism*. Mosaicism may also develop later in life when a chromosome abnormality develops in a stem cell line such as the Philadelphia chromosome abnormality of the myeloid stem cell line in patients with chronic myelocytic leukemia (Chapter 48). Two types of cells may also be found if stem cells from a twin or the mother have gained access to the fetus and, because of the fetal immunologic tolerant state, are permitted to grow, a circumstance termed *chimerism*.<sup>51</sup>

Chromosome abnormalities are classed as those of number, in which deviations from the *diploid* number of 46 occur, or those consisting of aberrant forms of individual chromosomes. Disturbances of number may reflect multiples of the normal *haploid* number of 23, in which instance the cell is



Fig 2.3 Normal male karyotype From the photograph of a metaphase spread (A) the chromosomes are arranged in groups according to their size (B) and the paired chromosomes are numbered consecutively, the male chromosome being designated as y and the female as x (Courtesy of Dr. Neil Wald)



*polyploid*. A cell which has lost (*hypodiploid*) or gained (*hyperdiploid*) one or more specific chromosomes so that an abnormal but not polyploid number is present is referred to as *aneuploid*. Aneuploidy is common in patients with acute leukemia, and polyploidy also may occur in these individuals (Chapter 46). The gain or loss of a single chromosome is found in patients with certain congenital syndromes such as the extra 21 chromosome (trisomy 21) in those with Down's syndrome or loss of one of the X chromosomes (monosomy) in those with Turner's syndrome. Abnormalities of chromosome appearance are varied and consist of such changes as an alteration in the position of the centromere from mid (metacentric) toward end (acrocentric) location, constriction of an area, or apparent loss of part of the chromosome. Missing portions of individual chromosomes may be *translocated* onto other chromosomes, rather than lost to the cell.

### Genetic Terminology and Patterns

Detailed consideration of each of the various inherited hematologic disorders will be presented in later sections but a general account of genetic terminology and patterns<sup>137</sup> is presented here.

Genes are the units of inheritance and are sections of DNA strands about which a number of modifying factors affecting phenotypic expression are clustered. They may be *structural* in which case the base sequences dictate protein structure, or *control* genes in which case the base sequences regulate the amount of product. A point mutation produces the structural abnormality of sickle hemoglobin (Chapter 25) and a mutation at a control point may produce thalassemia (Chapter 26). The site of the gene in the DNA strand is termed its *locus* and the corresponding gene on an homologous chromosome is termed its *allele*. Except for the X and Y chromosomes in the male which have no paired chromosomes, all genes have an allele on a paired *autosomal* chromosome.

A congenital disorder may be associated with genetic abnormality (Down's syndrome

with trisomy) and not be *inherited* (neither parent with the abnormality). Proof that the disease is inherited requires demonstration of the genetic defect in an accepted pattern within the family. Certain diseases such as hemophilia (Chapter 37) are inherited, but can also arise by spontaneous mutation. *Familial* defects can be defined as those in which the disorder seems to occur with abnormal frequency in a family but a genetic basis of inheritance has not been proved.

The term *heterozygote* is applied to patients with inherited diseases in whom a gene substitution is present at only one of the two alleles and the term *homozygote* to those in whom the gene is substituted at both alleles. The heterozygote inherits the abnormal gene from only one parent, the homozygote from both. The term *hemizygote* may be applied to a male who has inherited a sex-linked disorder on the X or Y chromosome. The term *double heterozygote* refers to a person who has inherited two phenotypically interacting but separate genetic abnormalities such as when an individual is heterozygous for both hemoglobin S and thalassemia.

Genotype refers to the gene composition of an individual and phenotype to the manner in which genotype is expressed. In a heterozygote there may be no phenotypic expression of an abnormal gene, which is then referred to as a *recessive* gene. A gene having strong phenotypic expression may be called *dominant*. In disease, the terms recessive and dominant are somewhat difficult to apply with any accuracy since by definition their use depends on the degree of sophistication used to study the phenotype. For example, most patients who are heterozygous for the sickle hemoglobin gene are asymptomatic (no superficial phenotypic expression, therefore the gene is recessive); however, if proper laboratory studies are carried out a phenotypic expression can always be detected (therefore the gene is dominant).

The concept of *expressivity* or *penetrance* underlies the use of the terms *recessive* and *dominant*. If the presence of the abnormal gene cannot be detected by any means of phenotypic examination it is said to be *non-penetrant*. Degree of penetrance in an indi-



vidual is modified by the rest of his genetic make-up and by environment.

Equal representation of gene expression for alleles in a pedigree is expected for all autosomal defects. However, defects carried on the sex chromosomes are variably expressed in the two sexes. Defects carried on the X chromosome such as hemophilia (Chapter 37) or primaquine sensitivity (Chapter 23) are expressed in the hemizygous male but will be variably expressed in the heterozygous female. The variable expression of females heterozygous for an X-linked gene may be partly explained by the *Lyon hypothesis*.<sup>124</sup> This suggests that, early in embryogenesis, either the maternally or paternally derived X chromosome in each cell becomes inactive and all descendants of such cells perpetuate that inactivity. This would explain, for instance, why some females heterozygous for hemophilia can be detected phenotypically while others cannot (Chapter 37). However, that such inactivation is not completely uniform is suggested by the observation that heterozygous females with phenotypically detectable hemophilia rarely if ever have defects as severe as their hemizygous sons.

Disease due to sex-linked inheritance involving the Y chromosome has not been detected in man.<sup>137</sup>

### Differentiation

The pattern of differentiation distinguishes one cell line from another since all cells in a normal individual bear the same chromosomal genetic pattern. In general it can be stated that just as DNA synthesis appears to be the key to proliferative activity, ribonucleic acid (RNA) synthesis appears to be the key to differentiation.<sup>42,50,58,87,120,153</sup> At least three classes of RNA molecules are recognized: high molecular-weight ribosomal RNA (rRNA), low molecular-weight transfer RNA (tRNA) with at least one type of molecule for each amino acid, and finally messenger RNA (mRNA). The mRNA molecules transmit the DNA coded message for synthesis of proteins. However, many factors regulate specific processes of differentiation, morphogenesis, and cellular organization.

Detailed knowledge of these is, to date, quite meager.<sup>42,175</sup>

### RNA Synthesis

RNA is transcribed from the DNA template under the influence of DNA-dependent RNA polymerases. The genetic code is followed by transcription of RNA adenine from DNA thymine, RNA uridine from DNA adenine, RNA cytosine from DNA guanine, and RNA guanine from DNA cytosine. Three consecutive base pairs of DNA make up a *codon* and each of the 64 possible codons carries instruction for a specific amino acid or for an instructional message to "start" or "stop" producing a polypeptide chain.

### Protein Synthesis

Protein synthesis is carried out in the cell by interaction of tRNA with mRNA, amino acids, various enzymes, and ribosomes. The *ribosomes*, composed of rRNA and protein, consist of two distinct units, one approximately twice as large as the other, which are joined together by magnesium ions and are the site of polypeptide chain synthesis and assembly. Amino acids are activated by an enzyme specific for each and react with specific tRNA to form amino-acyl-tRNA and then are bound to ribosomes.<sup>40</sup> Single-stranded mRNA moves across a sequence of ribosomes, forming an mRNA-polypeptidyl tRNA complex which is translocated to the polypeptidyl tRNA-binding site on the smaller ribosomal unit. Polypeptide assembly begins at the N terminal end and stops when a triplet coding for "stop" is reached in the mRNA sequence. The specific tRNA is released from the ribosome after its peptide has been added to the chain.

The *nucleolus* is thought to be a primary site of mRNA transcription and to play a significant role in production of ribosomal components.<sup>36,111,112</sup> Nucleolar synthesis appears to be under chromosomal control.<sup>102</sup>

Most ATP-trapped energy required for protein formation as well as that for other cellular events such as mitosis is generated by glycolysis or in the tricarboxylic acid

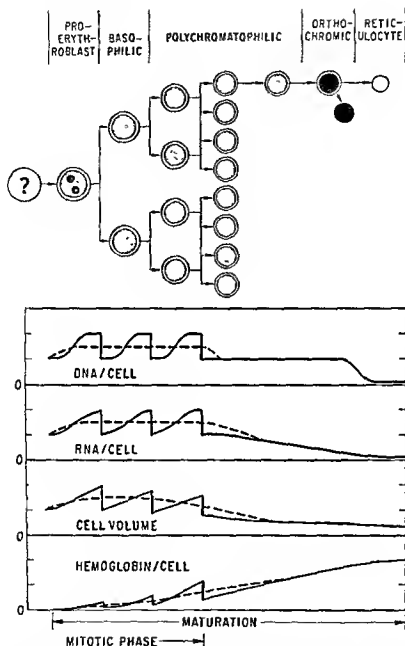


Fig. 2-4 Interrelation of proliferation and differentiation in the erythron. Erythropoietin triggers a stem cell into erythropoietic activity, perhaps by initiating hemoglobin synthesis. Subsequent maturation through identifiable morphologic stages is accompanied by cell division until midway through the polychromatophilic stage. Three successive cell divisions are illustrated but perhaps as many as six doubling divisions actually occur in the compartment (Chapter 4). During each generative cycle, DNA and RNA per cell double and are then reduced by half at mitosis. Cell volume increases during each cell cycle but does not double, leading to successively smaller cells. Volume contracts further during the process of postmitotic maturation. Cellular DNA, RNA content and volume are step functions in individual cells (solid lines in figure). Since the erythron is not a synchronized population measurement of these values as averages in the population would result in an integral and thus a smooth curve of change (dotted lines in figure). Hemoglobin synthesis persists throughout the mitotic and postmitotic maturation periods but RNA synthesis slows rapidly during the postmitotic period. Synthesis of DNA ceases during the postmitotic phase of the development cycle and most of the DNA is lost from the cell with nuclear extrusion.

cycle, usually in *mitochondria*. The mitochondria also are sites of important enzymatic reactions such as some of those involved in the biosynthesis of heme.<sup>121</sup> It should be noted that energy requirements seem to differ at different stages of the cell cycle. For instance, when energy is not generated, cells fail to enter prophase, but those in prophase complete mitosis.<sup>66</sup> The *Golgi apparatus* is a membrane-bound compartment of the cell containing enzymes involved in adding the more terminal sugar sequences to protein moieties, particularly those of membranes. Lysosomal granules are assembled at this site as are cell surface materials important in various recognition phenomena.<sup>220</sup>

### Interrelation of Differentiation and Proliferation

In certain developing hematopoietic cells, proliferation and maturation proceed concurrently for a period of time. In such systems the daughter cells of mitosis are presumably more mature than the mother cell at the beginning of the generative cycle. Proliferation ceases after a variable number of doubling divisions, but maturation continues. Under normal circumstances, erythrocytes (Chapter 4) and neutrophils (Chapter 6) are virtually mature by the time they enter the blood. However, other cells, such as monocytes (Chapter 6), continue the maturation process after traversing the blood and entering such tissues as the spleen, peritoneal cavity, and lung. An example of the interrelation of proliferation and maturation as it is seen in the erythroid compartment is shown in Figure 2-4.

## Hematopoietic Stem Cells

### Stem Cell Systems

As noted in the introduction to this section, each of the blood cells must be replenished from a stem cell compartment which remains stable in size under homeostatic conditions. In a stem cell compartment which remains stable in size but supplies differentiated cells, a cell must be added to the compartment by proliferation within the compartment for

each cell which leaves by the process of differentiation.<sup>171,221</sup> Osgood<sup>155</sup> suggested that such compartments are maintained by each cell division, resulting in one cell which leaves by differentiation and one which remains in the compartment (Fig. 2-5A), an asymmetric form of cell division. He termed

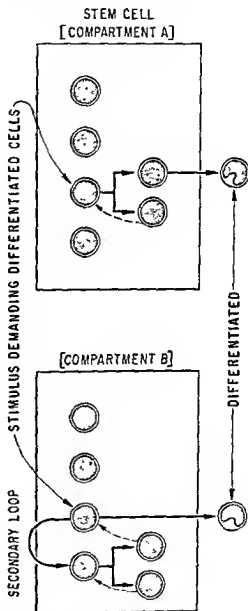


Fig 2-5 Models of stem cell replication. As discussed in the text, it has been suggested that division in stem cell compartments may be asymmetric (A) or symmetric (B). Compartment size is maintained in A by differentiating stimuli triggering cellular division in which one daughter matures and the other remains a stem cell. In B the differentiating stimulus depletes the compartment by inducing cellular differentiation, but a secondary "compartment depletion recognition loop" induces stem cell division to maintain compartment size.

this  $\alpha$ -N division and thought  $\alpha$ -2 $\alpha$  division in which both daughters remain stem cells to be an unusual event. An alternate model<sup>113,221</sup> separates the processes of differentiation and cell division and avoids the concept of asymmetric cell division. In such a model (Fig. 2-5B), for each cell which is triggered into differentiation and thus leaves the stem cell compartment, another is triggered into  $\alpha$ -2 $\alpha$  type division, thus maintaining a stable compartment size.

The exact structure and interrelationships of hematopoietic stem cell compartments are not known. On the basis of morphologic observations,<sup>111</sup> Maximow<sup>114</sup> and others considered it probable that all blood cells are derived from a common stem cell—the *monophyletic* theory. Others, such as Sabin and co-workers,<sup>184</sup> suggested that there is a separate and distinct stem cell compartment for each of the blood cells—the complete *polyphyletic* theory. Still other theories intermediate between these extremes (dualist, trialist, neo-unitarian, etc) were proposed by Naegeli, Schilling, Downey, and others (see Downey<sup>65</sup> and earlier editions of this book for a more complete discussion). Current evidence suggests that both the mono- and the polyphyleticists were correct.

Presently available data directly relating to stem cell compartments are derived mainly from studies of hematopoietic spleen colonies in the mouse,<sup>21-200</sup> colonies of granulocytic cells grown in vitro from marrow and blood of the mouse<sup>31</sup> and man,<sup>41,169</sup> or grown in vivo in diffusion chambers,<sup>30</sup> and from chromosomal or other cell changes associated with human diseases.<sup>185</sup>

Till and McCulloch<sup>209</sup> demonstrated a linear relationship between the number of marrow cells injected into lethally irradiated mice and the number of macroscopic nodules of hematopoietic tissue present on the surface of the recipient's spleen 10 days following the injection. Subsequent cytogenetic studies<sup>16,151</sup> disclosed that these colonies were *clonal*; i.e., they had arisen from a single cell. Such colonies may contain erythrocytes, neutrophils, megakaryocytes, and eosinophils, indicating that the colony-forming unit

cell (CFU cell) is pluripotent for the erythroid, neutrophilic, eosinophilic, and megakaryocytic cell lines. Cytogenetic data from patients with chronic myelocytic leukemia (CML) are compatible with the existence of such a cell in man.<sup>185</sup> When the Philadelphia chromosome abnormality (Chapter 46) is present in such patients it is demonstrable in precursors of erythrocytes, neutrophils, and eosinophils and probably in megakaryocytes but not in lymphocytes or buccal mucosa cells. It may also be present in marrow fibroblasts,<sup>126</sup> raising questions concerning a common fibroblast-hematopoietic stem cell. This suggests that CML is clonal in nature, beginning in a single cell which is pluripotent for the above four cell lines but not for lymphocytes. Other evidence supports the clonal nature of CML (Chapter 46). Similarly, in persons with paroxysmal nocturnal hemoglobinuria (Chapter 29) a membrane defect is present in erythrocytes, neutrophils, and platelets, thus suggesting a common origin for these cell lines.<sup>9</sup>

None of these studies throws light on the question of a common or separate origin of monocytes and basophils from neutrophils, eosinophils, or other cells. However, colonies grown in semisolid media from human marrow<sup>169</sup> or human blood<sup>44</sup> contain neutrophils, eosinophils, and monocytes. Since these colonies are clonal in nature, at least in man,<sup>46</sup> these observations in addition to the myeloblast-monoblast mixtures observed in patients with acute leukemia (Chapter 47) suggest a common origin for neutrophils, eosinophils, and monocytes.

In none of the above studies is there any suggestion that lymphocytes share a common precursor with other blood cells. Yet, under conditions of extreme cellular deprivation in the mouse, there is cytogenetic evidence that such a cell exists. In mice which recover from extreme depression of stem cell pools, a similar chromosome aberration was observed in hematopoietic spleen colonies and in lymphoid tissues.<sup>151,224</sup> The immune system of lethally irradiated mice has been repopulated with cells from spleen colonies.<sup>210</sup> In man, associated lymphocytic and erythrocytic de-

fects may be observed<sup>74</sup> and coexistence of congenital defects marked by marrow hypoplasia and hypogammaglobulinemia<sup>141</sup> are consistent with the concept of a cell pluripotent for myeloid and lymphoid tissue.

Lymphocytes appear to form a stem cell compartment under steady-state conditions. Small lymphocytes are capable of "blastic" transformation, cell division, and presumably reversion to small lymphocytes (Chapter 7).

The CFU cell appears to be in a state of  $G_0$  since high doses of tritiated thymidine or hydroxyurea (agents which kill cells in DNA synthesis, Chapter 55) do not kill an appreciable number of CFU cells when given to normal mice.<sup>35,176</sup> If the CFU compartment is not proliferating to any appreciable degree, it must be assumed that, under normal conditions, blood cell production is maintained from more mature stem cell compartments. Evidence for a more mature granulocyte and erythrocyte stem cell compartment has been presented. For example, the  $WW^v$  mouse has a genetic defect in CFU cells such that its marrow cells fail to produce macroscopic colonies in irradiated recipients. Yet, its marrow cells produce a normal number of normal-sized *in vitro* granulocytic colonies in soft agar.<sup>18</sup> Similarly, the size of the most mature stem cell system for erythrocytes, the "erythropoietin-sensitive cell" compartment, fails to parallel measured changes in the size of the CFU cell compartment, suggesting that these are separate cellular compartments.<sup>110,146,154</sup> Separation of cells from marrow or other tissues by various techniques tends to concentrate CFU cells, *in vitro* granulocytic colony-forming cells, and erythropoietin-sensitive cells into different fractions.<sup>93,143</sup>

In summary, present evidence favors a model for stem cell compartments (Fig. 2-6) with the following characteristics.

1. Differentiated stem cell compartments replenish blood cells under ordinary conditions. Neutrophils, eosinophils, and monocytes normally share a common compartment as judged by their coexistence in *in vitro* colonies.<sup>169</sup>

2. If the differentiated compartments are

damaged<sup>435,176</sup> or if an increased demand for mature cells is imposed,<sup>25</sup> a compartment normally in  $G_0$  begins proliferation and differentiation to replenish stem cell compartments for erythrocytes, megakaryocytes, neutrophils, eosinophils, and monocytes.

3. With extreme hematopoietic damage, a cell which is pluripotential for all blood cells is activated in order to replenish other stem cell systems.<sup>151,224</sup>

It is possible that these separate compartments are really a single compartment. Most studies which indicate their separateness rely upon demonstrating a difference in the proportion of cells in a generative cycle. An alternative explanation would be that there are differences in response to differentiation and differences in transplantability in association with changes in the cell cycle in a single stem cell compartment.

As yet, pluripotential stem cells cannot be identified. The small lymphocyte has been the prime candidate for the pluripotential cell since the time of Maximow<sup>134</sup> and, among others, Yoffey and co-workers<sup>143</sup> have favored the intermediate-sized lymphocyte (the transitional cell). Hematopoietic spleen colonies do not arise from transplanted lymph node, thymus, or thoracic duct lymphocytes, but can be obtained by transplanting cells from the marrow, spleen, blood, or peritoneal cavity.<sup>24</sup> Cells (presumably lymphocytes) responsible for graft-versus-host disease are separable from hematopoietic stem cells by sedimentation.<sup>168</sup> This indicates that the usual peripheral lymphocyte is an unlikely candidate for the pluripotential cell. However, cells with the morphologic appearance of lymphocytes are present in all of the above sources of colony-forming cells and thus the possibility of a "specialized" lymphocyte cannot be ruled out. Certain fractionation studies of marrow suggest that fractions concentrating lymphocytes also concentrate colony-forming cells.<sup>54</sup> However, gradient centrifugation also concentrates colony-forming cells in certain fractions which differ from the fractions containing small lymphocytes.<sup>93,143</sup> Colony-forming cells have been found in inflammatory exudates, and this and

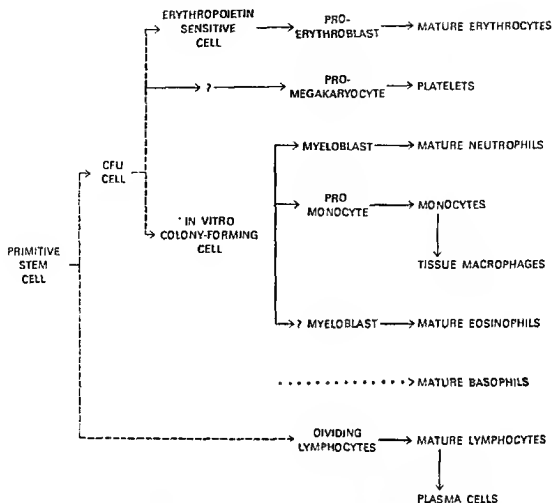


Fig 2.6 Interrelationship of blood cell origin. This diagram represents a possible structure of stem and mature cell compartments based on current evidence (see text). The most immature cell ("primitive" stem cell) is pluripotent for all blood cells. One of its progeny, the cell which forms spleen colonies in the mouse ("CFU" cell), is in turn pluripotent for all cells except the lymphocyte. Still more differentiated stem cells, such as the erythropoietin-sensitive cell, regularly maintain mature cell compartments. Solid lines represent pathways of regularly occurring cell flow in normal animals. Dashed lines represent pathways followed under circumstances of stress. The dotted line indicates the absence of data on basophil origin.

some ancillary evidence have been interpreted as favoring the monocyte as a colony-forming cell.<sup>13</sup>

The possible identity of the more differentiated stem cells will be considered in the chapters dealing with differentiation patterns of specific blood cells (pp. 181, 238, 298, 380).

### Regulation of Stem Cell Production

In general it can be stated that hematopoiesis is maintained in a steady state in which production of mature cells equals

cell loss. Increased demands for cells as a consequence of disease or physiologic change are met by increased cell production. Thus, the system must be subject to some form of feedback control. Such control could be exerted by humoral factors or on the basis of local cell concentration through cell-cell interaction.<sup>171</sup> There is evidence which suggests separate and distinct humoral control systems of erythrocytic (Chapter 4), neutrophilic (Chapter 6), and megakaryocytic (Chapter 9) tissue, but as yet no direct evidence of control of the hematopoietic system by cell-cell interaction has been presented.

Control of the size of the CFU compartment is even less well understood than are control mechanisms in differentiated cell compartments. The observation that irradiation of one limb leads to generalized proliferation of the compartment suggests humoral control.<sup>76</sup> However, attempts to demonstrate a CFU growth-promoting factor in mouse plasma have been unsuccessful to date.<sup>28</sup> If the entire CFU compartment is reduced in size, as by giving whole-body irradiation, self-replicative regrowth begins almost immediately.<sup>45</sup> Stimulation of mature cell production, as by injecting erythropoietin,<sup>131</sup> bleeding, or by injecting endotoxin,<sup>27</sup> leads to a prompt expansion of the CFU compartment. Thus, CFU cell proliferation is enhanced by reduction of compartment size or by increasing the demand for mature cells.

Differentiation, in the absence of self-replication,<sup>207</sup> is potentially suicidal for a stem cell compartment, particularly if the compartment has been reduced in size. An efficient protective mechanism may exist for the CFU compartment. After the compartment has been reduced to less than 10% of normal by irradiation, no differentiation can

be detected until regrowth by self-replication has partially restored the compartment.<sup>45</sup> The pathway of differentiation of CFU cells is geared to the degree of demand for such cells. Once differentiation begins in a reduced CFU compartment the relative output into erythroid or granulocytic lines is governed by the respective degrees of demand for each cell type.<sup>43,97</sup>

## Blood Formation in the Embryo and Fetus

Hemoglobin synthesis is initiated in the yolk sac in all embryonic vertebrates during the mesoblastic period of hematopoiesis.<sup>22,65</sup> Most investigators favor a mesenchymal rather than an entodermal origin for the blood islands of the yolk sac.<sup>98,204</sup> These cells produce unique hemoglobins (Fig. 2-7) not found during later erythropoiesis in any species yet studied<sup>14</sup> (see Chapter 4 for detailed discussion of hemoglobin types and structure).

The globin of the first detectable hemoglobin in the human embryo consists of

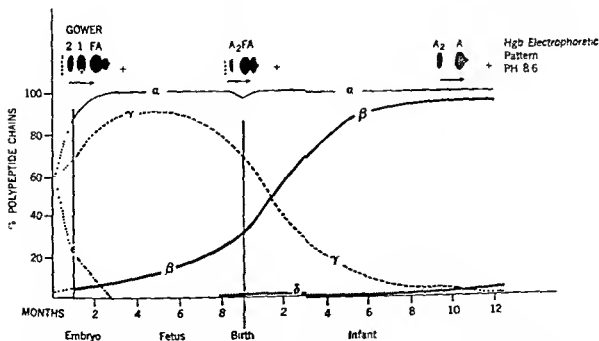


Fig 2-7. Proportions of the various human hemoglobin polypeptide chains through early life. The hemoglobin electrophoretic pattern (Gower-2, Gower-1, Hb-F, Hb-A, Hb-A<sub>2</sub>) typical for each period also is shown (From Pearson,<sup>163</sup> courtesy of author and C. V. Mosby Co)

polypeptide chains which differ from all other hitherto recognized globin chains and have been designated epsilon chains (Gower type I hemoglobin,  $\epsilon_1$ )<sup>96,100</sup> Shortly thereafter, production of normal alpha chains of hemoglobin begins and the yolk sac hemoglobin then can be represented as  $\alpha_2\epsilon_2$  (Gower type II hemoglobin) (Fig. 2-7) Insofar as has been determined, all circulating erythrocytes produced at this stage are large nucleated erythrocytes with immature-appearing nuclear chromatin. These cells are similar to and were regarded by Ehrlich, probably erroneously, as identical to the megaloblasts of pernicious anemia (Chapter 14) Demonstration of the unique hemoglobin in the yolk sac has led to the suggestion that these erythrocytes may be from a different cell clone than are later erythroblasts.<sup>109</sup> However, cells from the mouse yolk sac produce adult hemoglobin in culture.<sup>12</sup> Moore and Metcalf<sup>114</sup> demonstrated that migration of yolk sac stem cells was responsible for all subsequent hematopoietic development in the mouse. Thus, it appears that the unique  $\epsilon$  chains are formed in response to environment rather than being derived from a unique hematopoietic cell.

Beginning at approximately the sixth week of embryonic life, yolk sac production of erythrocytes decreases and production of erythrocytes within the human embryo begins. By approximately ten weeks, yolk sac erythropoiesis is undetectable.<sup>22,98</sup> Erythropoiesis is detectable in fetal liver by approximately six weeks, in fetal spleen by approximately 12 weeks, and in marrow by approximately 20 weeks.<sup>108</sup> By the time of full gestation, virtually all erythropoiesis is confined to the marrow.

Throughout fetal life, erythroblasts of liver, spleen, and marrow primarily produce fetal (F) hemoglobin ( $\alpha_2\gamma_2$ ) (see Chapter 4). It is not until after birth that appreciable conversion to adult hemoglobin is noted. In addition to Hb-F, cord blood contains two minor components (10%) designated  $F_1$  and  $F_{11}$ .<sup>4,150</sup> Nephrectomy and starvation fail to decrease neonatal hematopoiesis in rats,<sup>122</sup> but hypertransfusion results in suppres-

sion.<sup>225</sup> Hepatic erythropoiesis is stimulated by erythropoietin during certain stages but not during others.<sup>48,179</sup> These studies suggest that fetal hematopoiesis may proceed independently of erythropoietin or that erythropoiesis proceeds maximally at certain stages and cannot be further augmented. Production of fetal as well as adult hemoglobin was stimulated by erythropoietin in *in vitro* cultures of the marrow of newborn calves,<sup>78</sup> but no stimulation of fetal hemoglobin was demonstrable in similar culture of marrow of newborn human infants.<sup>149</sup>

Erythrocytes derived from yolk sacs are large (1800 to 2400  $\mu$ <sup>108</sup>) nucleated primitive cells<sup>22,134</sup> and have a shorter life span than adult erythrocytes, virtually all of them having disappeared by the fourth month. Later in fetal erythropoiesis, erythrocytes are smaller than yolk sac cells, but they still are macrocytic by adult standards. They generally lose their nucleus before entering the circulation.<sup>222</sup> Circulating red cells reach one million/ $\mu$ l between the second and third fetal month and then increase steadily to approach normal adult levels shortly before the end of normal gestation (Fig. 2-8).

Granulocyte precursors and megakaryocytes are demonstrable during later stages of yolk sac hematopoiesis, at least in some species.<sup>108,134,170</sup> (Fig. 2-9), but probably not in man.<sup>98</sup> Lymphocytes are not discernible (Chapter 7). Granulocytes and megakaryocytes are present during hepatic and splenic phases of hematopoiesis and megakaryocytes can be found in the circulation by 12 weeks.<sup>148</sup> However, it is not until hematopoiesis begins in marrow that granulocyte and megakaryocyte production becomes prominent. There is evidence in the mouse that the environment of the spleen is more favorable for erythrocyte than granulocyte production, as compared to the marrow.<sup>223</sup> Granulocytic stem cells are abundant in fetal mouse liver, but few granulocytes are formed there.<sup>178</sup> These data suggest that the marrow is the only potentially hematopoietic organ with the proper environment for full development of granulocytic and megakaryocytic tissue.



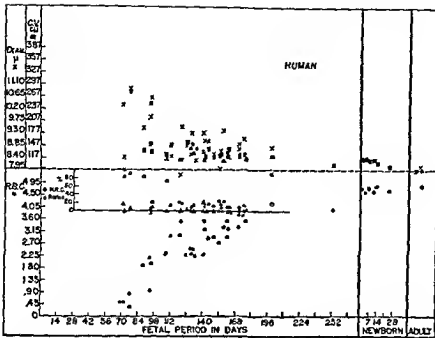


Fig. 2-8. The red corpuscles of human fetuses of different ages compared with those of the newborn and adult. Erythrocyte counts are represented by solid circles, mean corpuscular volumes by squares, mean erythrocyte diameters (measured in wet preparations) by crosses, proportion of nucleated red corpuscles by black triangles, and proportion of reticulocytes by open circles (From Wintrobe and Shumacker,<sup>222</sup> courtesy of the authors and American Society of Clinical Investigation, Inc.)

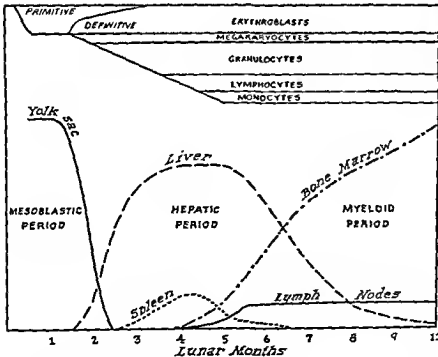


Fig. 2-9. Stages of hematopoiesis in the embryo and fetus, indicating the comparative participation of the chief centers of hematopoiesis and the approximate times at which the different types of cells make their appearance

## Blood of Newborn Infants and Children

The normal values for certain blood cells differ in infants and children compared to adults. Mauer<sup>133</sup> and Oski and Naiman<sup>161</sup> have reviewed these changes in greater detail than will be done here.

### Erythrocytes

The hematocrit of cord blood of full-term infants averages 52% (95% limits 47 to 57)<sup>55</sup> and there is little if any correlation between level of cord blood hemoglobin and birth weight or infant maturity.<sup>41</sup> In the few hours following birth the hemoglobin concentration in venous blood samples increases approximately 10%<sup>50, 51</sup> as does the hematocrit.<sup>62, 114</sup> This increase is probably due to infusion of cord and placental blood with subsequent reduction of plasma volume, as it is not observed if special attempts are made to clamp the cord early.<sup>62, 114</sup> Approximately

50 ml of placental blood are transferred to the newborn within the first minute after birth.<sup>211</sup>

At birth, as many as 24 nucleated erythrocytes per 100 leukocytes may be present in apparently normal infants, but these disappear by approximately four days.<sup>7</sup> As many as 5% reticulocytes are present at birth<sup>216</sup> and erythrocytes are quite macrocytic with an average mean corpuscular volume of 113 fl.<sup>68</sup> The life span of the red cells of the newborn is approximately three fourths that of the adult.<sup>83, 164</sup>

Erythrocyte production slows immediately after birth (page 69). By four days the hematocrit begins to decrease and continues to decline during at least the first two months of life and perhaps for longer periods.<sup>131, 149, 152</sup> The reduced erythrocyte production probably reflects two factors, both of which would result in decreased erythropoietin production (Chapter 4), namely, increased oxygenation of blood through the pulmonary as compared to the placental route and the increase in hematocrit produced by

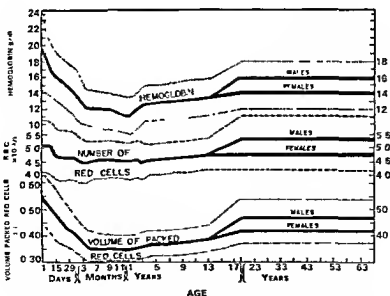


Fig 2-10. Normal curve for hemoglobin, red cells, and volumes of packed red cells from birth to old age. The mean values are heavily outlined. The range of variation is indicated by dotted lines for hemoglobin, interrupted lines for red cell count and dotted interrupted lines for volume of packed red cells. The scales for hemoglobin, red cell count and volume of packed red cells are similar and therefore the relative changes in these three values are apparent on inspection. The scale for age, however, is progressively altered.

infusion of cord and placental blood. Erythropoietin can be detected in the plasma of the newborn and then is undetectable for two to three months.<sup>129</sup> This does not reflect inability to produce erythropoietin since it is increased in the plasma of anemic or hypoxic infants.<sup>90</sup> Evidence for an inhibitor of erythropoiesis in neonatal plasma has been presented.<sup>196</sup> Additional factors contributing to the decline in hematocrit observed after birth are hemolysis and an expanding plasma volume.<sup>152</sup> The "physiologic anemia" of newborns may be more severe in premature infants than in full-term infants. In prematures, "normal" hemoglobin levels have been reported to be as low as 6 g/100 ml at the age of three to seven weeks.<sup>152</sup> In full-term infants, minimal hemoglobin values are approximately 9 g/100 ml at the age of seven to nine weeks.<sup>132</sup>

Exact normal values for erythrocytes in infancy and childhood are difficult to establish<sup>77</sup> owing to the frequency of iron deficiency in infants (Chapter 17). However, following the postnatal decline, average values remain below those of adults until teenage is reached (Fig. 2-10). The macrocytosis present at birth disappears by three months.<sup>61,132</sup> Between the ages of five months and three years, minimal normal values of 10.5 g per 100 ml hemoglobin and 34% hematocrit seem reasonable.<sup>86,91,132,133</sup> Total blood volume correlates reasonably well with linear height of children.<sup>52</sup> By puberty, values for boys and girls are equal to or perhaps exceed those found in adult women.<sup>81,133</sup> In 471 healthy children living near sea level, aged 11, 12, or 13, we found mean VPRC as determined in the Wintrobe hematocrit to be 44 ml/100 ml (95% confidence limits 41 to 49) and values in boys and girls were identical.

### Blood Leukocytes

*Neutrophil concentration* (Fig. 2-11) is quite high at birth and may rise even higher during the first days of life. At birth there is a moderate shift to the left; metamyelocytes are easily found and myelocytes and even

promyelocytes may be observed.<sup>106</sup> Within two weeks, neutrophil concentration declines to within, and occasionally below, normal adult levels. The neutrophil concentration in children four years of age or older is the same as in adults.<sup>157-160</sup> *Lymphocyte concentration* is high at birth and rises to values as high as 22,000 in some apparently healthy infants during the first year of life.<sup>215</sup> A gradual decline is observed throughout childhood, but even teenagers have higher concentrations than adults.<sup>157-160</sup> *Eosinophil concentration* may be rather high during the first year of life and tends to be higher in children than in teenagers or adults. *Monocyte* levels are high during the first year but thereafter are at adult levels. No significant relation of age to *basophil* concentration has been detected.

### Platelets

The platelet count of normal, full-term infants is similar to levels in adults.<sup>1</sup> There is disagreement in the literature as to whether otherwise normal premature infants commonly<sup>139</sup> or uncommonly<sup>71,105,165</sup> are thrombocytopenic. Platelet counts appear to be maintained at normal adult levels throughout infancy and childhood.

## Extramedullary Hematopoiesis

Formation of apparently normal blood cells outside the confines of marrow has been noted in post-fetal life under a variety of circumstances (Chapter 57). The spleen is the most commonly encountered site, but hematopoiesis in liver, lymph nodes, and, less commonly, adrenal glands, cartilage, broad ligament, thrombi, adipose tissue,<sup>75</sup> intrathoracic areas,<sup>49</sup> kidney, and endostium<sup>32</sup> also has been recorded. These hematopoietic islands may be composed of apparently pure or mixed erythrocytic, granulocytic, or megakaryocytic tissue, reminiscent of the clonal spleen colonies of the mouse recovering from irradiation (page 50).

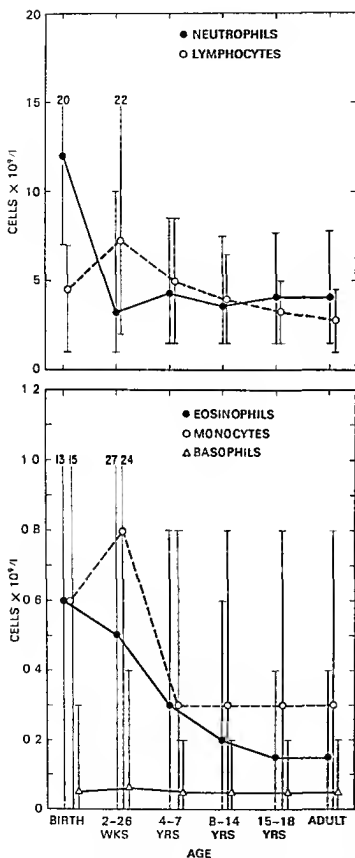


Fig. 2-11. Values for blood concentration of neutrophils and lymphocytes (above), and of monocytes, eosinophils and basophils (below) during infancy and childhood (Compiled from the data of Kato<sup>106</sup> and Washburn<sup>215</sup> for infants and from data of Osgood and co-workers<sup>137 138 139 160</sup> for older children)

In general, extramedullary hematopoiesis has been found in association with diseases characterized by increased production of one or more types of cell (erythroblastosis fetalis,<sup>32</sup> pernicious anemia,<sup>123,140</sup> thalassemia,<sup>198</sup> sickle cell anemia,<sup>191</sup> hereditary spherocytosis,<sup>49</sup> and various leukemias<sup>104,186</sup>). Chronic bleeding or chronic poisoning of cell production will produce extramedullary hematopoiesis in animals.<sup>83</sup> However, increased demand for cells in the face of decreased production does not necessarily lead to extramedullary cell production. Aplastic anemia is rarely associated with extramedullary hematopoiesis.

In most of the above instances, abnormally immature cells are found in the blood. As is discussed on page 60, cell release even from normal marrow is not understood. It is possible that the factors inhibiting the release of immature cells from normal marrow are not operative at extramedullary sites.

## Medullary Hematopoiesis

In normal adults, marrow which is active in hematopoiesis is limited to the vertebrae, ribs, sternum, pelvis, scapulae, skull, and extreme proximal portions of the humeri and femora. The marrow cavity of the remaining bones of the limbs is filled with fat. Under abnormal conditions characterized by long-standing increased hematopoiesis, a peripheral expansion of active hematopoietic marrow may be observed.<sup>130</sup> In infants and children, active hematopoiesis takes place in more distal portions of the extremities than in adults. Crude estimates of the location of active hematopoietic marrow can be obtained by injecting appropriate isotopes and scanning the entire body for radioactive emissions.<sup>3,212</sup> Labeled colloids are phagocytosed by the reticuloendothelial cells of the marrow and in the normal state their distribution is equal to the distribution of active hematopoiesis. However, in the presence of disease the extent of active marrow estimated by means of labeled colloids may differ from that estimated by radioactive iron.<sup>212</sup>

## Anatomy of the Bone Marrow

The marrow cavity of the bones of man is partially compartmentalized by plates of bone trabeculae protruding into the cavity at right angles to the external medullary bone. Hematopoietic marrow contained within this space is a loosely knit gelatinous to semifluid tissue rich in fat.

The vascular system of the marrow is complex and hematopoietic cell production appears to follow the vascular arrangement rather exactly.<sup>59,219</sup> Centrally located nutrient arteries coursing through the marrow cavities send out branches which terminate in capillary beds within the bone or, less frequently, at the periphery of the marrow space. Certain of the capillaries or post-capillary venules (vessels of the osteal canals) reenter the marrow cavity and coalesce to form large, venous sinuses in which the somewhat sluggish flow is again toward the center of the cavity. These sinuses often have a complex pattern of intercommunication, but eventually flow into the central vein, which follows the same general course as the central artery. Hematopoietically active parenchyma and fat fill the space between the sinusoids. Light and electron microscopy indicate that hematopoiesis takes place outside,<sup>59,219</sup> rather than within,<sup>153</sup> marrow sinusoids. Blood flow to marrow is rapid, calculated as 0.5 ml/g of marrow tissue/min in rabbit femurs.<sup>55</sup>

The wall of the venous sinusoids of the marrow is primarily composed of a unicellular endothelial cell network.<sup>60,219</sup> In other sinuses, such as those of the spleen (Chapter 8), two additional components of sinusoidal walls are regularly present, a basement membrane and an adventitial cell layer. In marrow sinuses, these components are intermittently found but they are so discontinuous that the endothelial layer constitutes the only continuous component. When a basement membrane is found it is usually at a location where a muscular artery lies close to the endothelial layer.<sup>60</sup> Fenestrations and areas of discontinuity of the endothelial lining have been reported.<sup>219</sup> However, others<sup>354,60</sup> have described the membrane as being continuous

fenestrations occurring in the endothelial cell itself only when that cell is penetrated by an emerging blood cell. Whether fenestrations normally exist or only develop with trans-endothelial passage of blood cells is an important question with respect to the mechanisms of release of cells from marrow (see below). Adventitial cells extend into the hematopoietically active sections with their cytoplasmic membrane fitting in between and conforming to the contour of hematopoietic cells. The "proteinaceous" material noted between hematopoietic cells in light microscopy is adventitial, or endothelial, cell cytoplasm according to some electron microscopy studies.<sup>219</sup> *Adventitial cells are classed as reticular cells* (Chapter 8), that is, they are considered to possess the capacity to form reticular fibers and to be phagocytic. As a rule, little phagocytic activity is noted in endothelial cells. The proposal that these cells may serve as stem cells for hematopoiesis<sup>134</sup> has received no substantiation. However, when parenchymatous marrow is mechanically removed from the bone of an animal, regeneration may be from residual local stem cells in the bone rather than from circulating cells<sup>125</sup>; a similar pattern has been reported in studies of extramedullary marrow implants.<sup>128</sup> The type of cell leading to this regeneration is unknown, but there is evidence that osteoblasts and hematopoietic cells arise from separate stem cells.<sup>6</sup> The fat cells of marrow primarily represent accumulation of fat within adventitial cells, although endothelial cells may also accumulate fat.

Hematopoiesis occurs within the parenchyma of marrow, bounded by the sinusoids. In both light and electron microscopy, specific types of cells in various stages of development tend to aggregate. That is, the impression is gained that a small island of pure erythropoiesis abuts a similar neutrophil island and that cells are not scattered at random.

#### Release of Cells from the Bone Marrow

The control of cell release and the exact mechanisms of release are incompletely un-

derstood. There seems no doubt that cells leave the parenchyma and enter the sinusoids through fenestrations of the endothelial cells lining the sinuses, but how these fenestrations are created is not clear. As already noted, there is disagreement as to whether endothelial fenestrations normally exist in the absence of migrating blood cells.

Since granulocytes are motile and become more motile and more deformable<sup>119</sup> as they mature, it has been assumed that they migrate directionally toward the sinusoid. One hypothesis to explain the action of neutrophil-releasing factor<sup>26</sup> would be to suggest that this factor acts as an attractant for mature neutrophils.

Megakaryocytes usually are observed in close proximity to the sinusoidal membrane, and cytoplasmic processes fenestrating endothelial cells have been described.<sup>60,219</sup> Thus, direct release of platelets into the sinusoid through cell rupture or theoretically through shedding of cytoplasm requires only disruption of the megakaryocyte cytoplasmic membrane (Chapter 9).

The mechanism underlying release of the basically nonmotile erythrocyte is unknown. In response to acute hemorrhage, a sudden increase in the number of mature erythrocytes in marrow parenchyma is observed.<sup>219</sup> This suggests that certain vessels may empty directly into the parenchyma and under certain circumstances may "wash" cells from the parenchyma into the sinuses. Sinusoidal dilatation with attendant increased blood flow accompanies hypoxia.<sup>57</sup> Adventitial cells can shift position in response to enlarging sinuses, perhaps compressing the parenchyma and "squeezing" out cells by contracting the parenchymal space.<sup>219</sup> The viscosity of "intercellular" materials of marrow parenchyma has been suggested as a means of influencing erythrocyte release. Induction of erythroid hyperplasia in rabbit marrow led to decreased viscosity; granulocytic hyperplasia caused increased viscosity.<sup>39</sup> However, as noted earlier, what appears to be "intercellular" material by light microscopy has been suggested to be adventitial cytoplasm by electron microscopy.

One important factor in cell release appears

to be cell deformability. Reticulocytes and mature red cells<sup>217</sup> and granulocytes<sup>119</sup> are much more deformable than are their precursors. Perhaps release is controlled by a combination of changing blood flow directly into parenchyma, sinusoid and parenchymal volume, and levels of cell attractants in sinusoidal blood. Whether a cell is actually released may depend upon its ability to deform enough to traverse the fenestrations of the sinusoidal wall.

Accelerated platelet or neutrophil release can occur as isolated phenomena. Accelerated release of red cells usually is accompanied by accelerated release of other elements. For instance, following acute hemorrhage or with a brisk hemolytic anemia, the early release of reticulocytes usually is accompanied by accelerated neutrophil and platelet release (Chapter 4). However, with accelerated platelet release in certain thrombocytopenias (Chapter 34) or accelerated neutrophil release (Chapter 6), accelerated release of other cells usually is not seen. These observations can be interpreted as suggesting specific humoral releasing factors for platelets and neutrophils.<sup>28</sup> Red cell release, on the other hand, appears to depend more on mechanical factors.

Diseases of marrow structure such as myelofibrosis or carcinomatous invasion (Chapter 57) could disrupt areas of sinusoidal architecture. In leukemia and related diseases (Part V, Section 3) the expanded parenchyma itself may possibly change the size of sinusoidal fenestrations. Certainly, in most circumstances in which immature cells are found in the blood, there is evidence for some change in marrow structure or there is extramedullary hematopoiesis.

#### Number of Hematopoietic Cells in Bone Marrow

Estimates of the total number of hematopoietic cells within the marrow cavities of man have been made by a variety of techniques. The rate of plasma iron turnover (Chapter 4) bears a direct relation to the total number of nucleated erythrocyte precursors in the marrow of normal man as well as in

patients with various diseases.<sup>70a</sup> The derived value is approximately  $3.5 \times 10^9$  normoblasts/kg in normal man. Considering the usual ratio of three nonerythroid to one erythroid cell (Table 2-1) this suggests that there is an average of approximately  $14 \times 10^9$  nucleated marrow cells/kg. Donohue and co-workers<sup>64</sup> studied radioactive iron localization in marrow of ribs removed at thoracotomy and calculated an average of  $18 \times 10^9$  nucleated marrow cells/kg. Total marrow mass can be calculated from the rate of turnover of mature blood cells if the relation of turnover to the number of marrow precursor cells has been established (see Chapter 6 for an example of calculation of neutrophil mass). Harker<sup>93</sup> carried out such studies for platelets and megakaryocytes and arrived at figures which correlated well with those given above. By making a variety of assumptions concerning the structure of marrow compartments for neutrophils, a figure of  $18 \times 10^9$  nucleated marrow cells/kg was derived.<sup>23</sup>

#### Weight of Bone Marrow

It has been calculated from autopsy studies that the weight of marrow tissue approximates 3.4 to 5.9% of total body weight in the adult or 1600 to 3700 g.<sup>138</sup> Thus, the weight of marrow is roughly equivalent to that of the liver.

#### Functional Capacity of the Bone Marrow

A very large increase in production of all marrow cells can be induced not only by diseases such as polycythemia vera (Chapter 30) but also in response to increased cell demands fostered by excessive cell loss as is observed in patients with hemolytic anemia (Chapter 21), chronic infection (Chapter 42), or idiopathic thrombocytopenic purpura (Chapter 34).

Expanded production can be accomplished by increased parenchymal cellularity with loss of fat in areas of normal hematopoiesis,<sup>39</sup> expansion of hematopoietic marrow into previously inactive fatty marrow cavities, short-

**Table 2-1. Differential Counts of Bone Marrow Aspirates from 12 Healthy Men**

	Mean (%)	Observed Range (%)	95% Confidence (%)
<b>Neutrophilic series (total)</b>	<b>53.6</b>	<b>49.2-65.0</b>	<b>33.6-73.6</b>
Myeloblast	0.9	0.2-1.5	0.1-1.7
Promyelocyte	3.3	2.1-4.1	1.9-4.7
Myelocyte	12.7	8.2-15.7	8.5-16.9
Metamyelocyte	15.9	9.6-24.6	7.1-24.7
Band	12.4	9.5-15.3	9.4-15.4
Segmented	7.4	6.0-12.0	3.8-11.0
<b>Eosinophilic series (total)</b>	<b>3.1</b>	<b>1.2-5.3</b>	<b>1.1-5.2</b>
Myelocyte	0.8	0.2-1.3	0.2-1.4
Metamyelocyte	1.2	0.4-2.2	0.2-2.2
Band	0.9	0.2-2.4	0.2-2.7
Segmented	0.5	0-1.3	0-1.1
<b>Basophilic and mast cells</b>	<b>&lt;0.1</b>	<b>0-0.2</b>	
<b>Erythrocytic series (total)</b>	<b>25.6</b>	<b>18.4-33.8</b>	<b>15.0-36.2</b>
Pronormoblasts	0.6	0.2-1.3	0.1-1.1
Basophilic	1.4	0.5-2.4	0.4-2.4
Polychromatophilic	21.6	17.9-29.2	13.1-30.1
Orthochromatic	2.0	0.4-4.8	0.3-3.7
<b>Lymphocytes</b>	<b>16.2</b>	<b>11.1-23.2</b>	<b>8.8-23.8</b>
Plasma cells	1.3	0.4-3.9	0-3.6
Monocytes	0.3	0-0.8	0-0.6
Megakaryocytes	<0.1	0-0.4	
Reticulum cells	0.3	0-0.9	0-0.8
<b>M:E ratio</b>	<b>2.3</b>	<b>1.5-3.3</b>	<b>1.1-3.5</b>

ening of cellular maturation time, and perhaps by accelerating generation time. In general, production can be accelerated to a much greater degree in response to a long-standing chronic stimulus than to an acute stimulus.<sup>53</sup>

In rabbits with chambers implanted in bone the following sequence of conversion from nonhematopoietic to hematopoietic marrow has been observed: resorption of some bony trabeculae, growth of arterioles into the cavity from bone, sinus formation, and finally formation of islands of hematopoiesis.<sup>135</sup> In man, increased cell production as observed in association with leukemia, is accompanied by a selective increase in blood flow to the marrow.<sup>167</sup>

Erythrocyte production has been calculated as six to twelve times normal in persons with congenital spherocytic hemolytic anemia (Chapter 21). Platelet production has been calculated as being as high as eight times

normal in those with idiopathic thrombocytopenic purpura (Chapter 34). Neutrophil production may be four times normal in patients having chronic infection (Chapter 42). The relative importance of the various mechanisms for increasing cell production mentioned above has not been determined, but an increase in the total number of precursors appears more crucial than shortening of stem cell to mature cell maturation time.<sup>91</sup> Temperature of marrow cavities (which would presumably in part be secondary to blood flow) has been suggested as a factor regulating fatty versus hematopoietically active marrow.<sup>57</sup> While it is true that marrow activity is favored when rodent vertebrae are transplanted from the tail (cold) to the peritoneal cavity (warm), it appears that physiologic stimulation, as with high erythropoietin levels, is required to produce conversion from fatty to hematopoietically active marrow.<sup>127</sup>

Increased production of one cell line is not



accomplished at the expense of another except under unusual experimental circumstances. In mice recovering from irradiation with a consequent reduction in the size of the stem cell pool there may be "competition" for available stem cells.<sup>43,97</sup> In such animals, if an abnormal demand for erythrocytes (bleeding, erythropoietin injection) is imposed, granulocyte production is decreased even further; the converse is true in plethoric animals. However, with normal stem cell pools, simultaneous demands for increases in output of various cell lines can be met. For instance, after normal animals are bled, red cell, neutrophil, and platelet production are increased simultaneously.<sup>84</sup>

## Methods of Obtaining Bone Marrow Specimens

Normal bone marrow is soft and semifluid during life and consequently can be removed for examination by aspiration as well as by biopsy techniques. Marrow biopsy was performed in 1903 by Pianese, who punctured the epiphysis of the femur by means of a trocar, and in 1908 by Ghedini, who trephined the tibia in its upper third.<sup>190</sup> In 1923, Seyfarth<sup>190</sup> trephined the sternum, choosing it because of accessibility, the thinness of the bone, and the likelihood of finding active marrow at this site throughout life. Needle aspiration of the bone marrow was proposed by Arinkin<sup>8</sup> in 1929 and since that time this procedure has gained preference over open surgical biopsy. Open surgical bone marrow

biopsies have now been largely supplanted by needle biopsy when information beyond that which can be obtained by examination of aspirated material is desired (page 75). A number of monographs dealing with the subject of bone marrow study have been published.<sup>29,56,116,177,189,190,193</sup>

### Aspiration of Bone Marrow

Many types of needles have been used for marrow aspiration. Needle gauges ranging from  $\#14$  to  $\#18$  have been fully satisfactory in our experience. Compared with the larger needles, the smaller ones have the slight advantage of easier penetration of bone, but the disadvantage of being bent or deformed more easily while penetrating the bone. Adjustable guards which limit the depth of penetration may be desirable for operators without extensive experience in marrow aspiration. A satisfactory needle is shown in Figure 2-12.

Various sites may be used for puncture and aspiration. In adults, all things being equal, the sternum at the second intercostal space is the most satisfactory. The likelihood of obtaining a satisfactory cellular aspiration is greater at this site than from the iliac crest or other areas, and the thinness of the sternum adds to its suitability. Aspiration should not be attempted from the body of the sternum below the second intercostal space. At lower levels the great vessels and right atrium present a hazard. If one has the misfortune of penetrating the inner table, severe or even fatal hemorrhage and/or pericardial tampon-

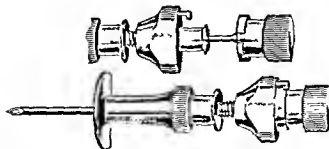


Fig 2-12. University of Illinois Stereal Needle with adjustable guard, special locking device for the stylet and Luer-Lok hub set into the needle. (Courtesy of V. Mueller and Co., Chicago)

ade may result.<sup>10</sup> To our knowledge these are the only causes of mortality reported from needle aspiration or biopsy of the marrow. We have observed serious infections in neutropenic patients at the site of open surgical biopsy but rarely at sites of needle aspiration or biopsy. The space behind the second intercostal space contains fat and lymph nodes primarily. In the unfortunate event of mediastinal penetration, some degree of mediastinitis or pneumomediastinum is the worst complication that can be expected.

The operator should scrub prior to aspiration and puncture as for any surgical procedure and in some instances he should wear surgical gloves. To exceedingly apprehensive patients, sedation may be administered in advance of the procedure. The area of skin to be punctured is washed, shaved if considered necessary, an antiseptic is applied, and sterile towels are laid closely around the site. The skin and the periosteum must be anesthetized if severe pain is to be avoided. Since the tissue lying between skin and periosteum is virtually devoid of painful nerve endings, infiltration of this intervening tissue is unnecessary. With a hypodermic needle of approximately 25 gauge, the most superficial layer of skin is infiltrated with procaine; the needle is next advanced until the bone is touched. At this point, procaine is injected in order to obtain anesthesia of the periosteum. If a guard regulating the depth of penetration is to be used, the distance required to reach the sternum by the infiltrating needle is measured and an additional distance is added to include penetration of the outer table of the sternum. This outer table varies considerably in thickness, ranging from 0.2 to approximately 5 mm.

The marrow aspiration needle is passed vertically, with a slight rotating or boring motion, into the sternum between the second and third ribs in the midline or slightly to the side of the midline. Rotation of the needle aids penetration; a "give" is felt when the marrow cavity is entered. The cavity is normally 5 to 15 mm in depth which permits the needle to be passed another 1 or 2 mm after the "give" has been felt.

When the marrow cavity has been entered, the stylet is removed from the needle and a sterile syringe is attached. The size of syringe is unimportant as long as it is well fitting, thus providing significant pressure for aspiration. The plunger is slowly withdrawn until the first drop of marrow appears in the syringe. The syringe should be removed at this point and, if for any reason a larger volume of marrow is desired, this should be collected in a second syringe. The patient usually experiences momentary pain when suction is applied, provided the needle is in the marrow cavity. If no marrow is obtained the needle should be successively rotated, advanced, and retracted, and suction again applied. If this proves unsuccessful, it is probably best to try another site. Pressure for a few moments over the site of aspiration usually obviates any bleeding from the puncture site. However, if bleeding does occur, a tight dressing should be applied.

Other sites satisfactory for aspiration of the marrow include the iliac crest, posterosuperior iliac spine, spinous process, rib,<sup>115,177</sup> and, in infants, the head of the tibia. The same procedure outlined for the sternum is followed when any of these sites is selected. For aspiration from the iliac crest, the anterosuperior spine of the crest should be identified by palpation and a site for aspiration chosen 1 to 2 inches posterior to this and just under the palpable lip of the crest. The needle is then directed so as to penetrate the crest area from below the lip. For posterosuperior iliac spine aspiration, the needle is directed vertically into the center of this oval protuberance (Fig. 2-13). For spinous processes, the third or fourth lumbar vertebra usually is chosen and the patient may assume a sitting position if desired. The needle should be directed so as to anticipate following the axis of the spinous process. In children under two years of age, the flat triangular area at the proximal end of the medial surface of the tibia, just below the tibial tubercle and medial to the tibial tuberosity, may be punctured. However, even in young children the posterosuperior iliac spine or the iliac crest is quite satisfactory. Because ster-

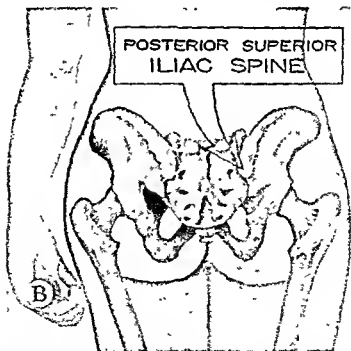
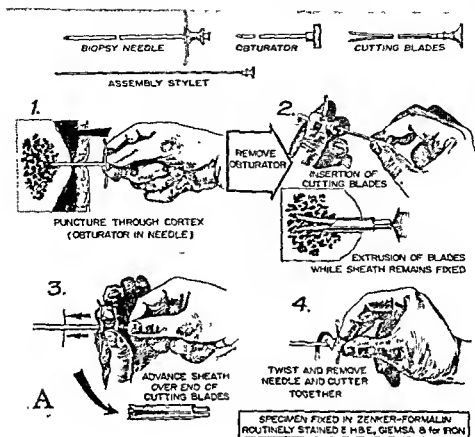


Fig. 2-13. Westerman-Jensen technique for needle biopsy of bone and marrow. A, Biopsy needle and outline of steps in its use. B, site of biopsy (From Ellis, L. D. et al.,<sup>67</sup> courtesy of authors and American Medical Assn.)

nal aspiration often frightens young children it should be avoided.

Inherent in considering alternate anatomic sites as suitable for aspiration is the assumption that active marrow contains the same proportion of cells in all anatomic sites. Comparison of sternum, ribs, vertebrae, femur,<sup>116-199</sup> and iliac crest of adults,<sup>181</sup> and sternum, tibia, femur, and vertebrae of infants<sup>80,209</sup> suggests that this assumption is correct in normal subjects.

### Biopsy of Bone Marrow

When aspiration of marrow provides inadequate material ("dry tap") or in certain clinical situations in which one may anticipate additional information when a larger specimen is obtained (page 75), biopsy should be performed. Such biopsies are carried out by using various modifications of the Vim-Silverman needle<sup>136</sup> such as that of Westerman and Jensen.<sup>67</sup> Needles with larger bores or with multiple openings are also available.<sup>142-172</sup> A needle designed to avoid "crush artifact" which is sometimes a problem with the above needles has been described.<sup>101</sup> The Westerman-Jensen needle is supplied with finger grips, an assembly stylet, and an obturator which locks in position (Fig. 2-13A).

The posterosuperior iliac spine is the most commonly used site for biopsy. The patient is placed in a lateral recumbent position. The needle, with the obturator locked in place, is inserted into the previously anesthetized skin overlying the posterosuperior iliac spine (Fig. 2-13B). It is inserted through the skin and bone cortex by using a rotating movement. Once the medullary cavity has been reached, the stylet is unlocked and removed. The cutting blades, with the assembly stylet in place, are inserted into the outer cannula, the assembly stylet is removed, and the blades are advanced until the medullary bone has been entered. The cutting blades are pressed into the medullary bone while the outer cannula is held firmly in a stationary position. The outer cannula is then advanced over the cutting blades, thus trapping the tissue, and the entire unit is then removed. The specimen

is separated from the cutting blades by teasing it from the tip before withdrawing the blades through the cannula.

Optimal specimens are approximately  $\frac{3}{16}$  inch long,  $\frac{1}{16}$  to  $\frac{1}{8}$  inch in diameter, and have wet weights of about 150 mg. Cultures, imprints ("touch preparations"), and histologic sections are prepared, as discussed below.

Post-biopsy care ordinarily consists of applying pressure over the posterior ilium for about 60 minutes. Pressure is obtained by means of a pressure dressing and by having the patient lie recumbent in bed. Patients with a bleeding tendency or other complications are carefully observed for a longer period. Analgesics are seldom necessary after the procedure.

## Preparation of Bone Marrow Specimens

### Smears

The best smears are made by placing a macroscopic marrow particle from the aspirate between scrupulously cleaned coverslips, squashing the particle between the coverslips with very slight pressure, and then pulling the coverslips gently apart so that the particle is smeared onto both of them. For this purpose, the small amount of material aspirated into the first syringe may be expressed onto a watch glass or onto a slanted glass slide and the visible particles picked up with either a pipet or a corner of a coverglass. If particles are not visible in the aspirated material, a small drop of the material should be placed between the coverslips and prepared as one would prepare a blood smear (Chapter 1). Smears from the aspirated material must be prepared promptly to avoid clotting, although they may be prepared more leisurely if the aspirate has been anticoagulated. However, all available anticoagulants introduce some artifactual change in the structure of marrow cells. Of those presently available, ethylenediamine tetraacetic acid (EDTA) introduces fewer artifacts and thus is the anticoagulant of choice. Slides may be stained with Wright's stain or with a May-Grun-

wald-Giemsa stain, depending on individual preference. The routine use of an iron stain, such as the Prussian blue, for marrow smears also is recommended (page 628). In addition, one may wish to stain for peroxidase or alkaline phosphatase.

### Supravital Preparations of Marrow

Supravital staining offers an opportunity to study motile cells that are partially stained. On slides prepared with a dry film of stain, a portion of marrow, preferably a macroscopically visible particle, is flattened under a coverglass by gentle pressure. The coverglass is then sealed along its edges to the underlying slide with petroleum jelly. The living cells can then be observed. When examined by skilled observers, this preparation in conjunction with the study of smears stained with Wright's stain may provide added information, as, for example, in differentiating types of acute leukemia. (Chapter 47).

### Concentrated Preparations<sup>19</sup>

When few cells are found in the smears made from freshly aspirated specimens, it may prove useful to concentrate cells from an anticoagulated portion of the aspirate. This marrow can be centrifuged in tubes of small diameter such as the Wintrobe hematocrit tube. Fat, plasma, nucleated cells, and red cells separate into visible layers and smears are prepared from the nucleated cell layer. As previously noted, anticoagulation may lead to cell artifact. Consequently, subtle changes on such smears must be interpreted with caution. Furthermore, since cells of different density sediment in different regions of the nucleated cell layer, the differential count may be inaccurate.

### Preparation of Sections from Biopsy Specimens

Various means for making sections from clots or material concentrated from aspirated marrow have been described.<sup>5,19,20,21,157,158</sup> These sections often provide information be-

yond that obtained by examination of smears.<sup>118</sup> However, with the introduction of relatively simple means of obtaining a needle biopsy specimen (Fig. 2-13), if sections are desired, this procedure rather than section of clots obtained by aspiration should be employed. Biopsy specimens are placed directly in Zenker's acetic acid solution and processed in routine fashion prior to staining. Staining with hematoxylin and eosin, with Giemsa, and with iron stains is carried out routinely. Modified thin-section preparation methods such as that of Block and associates<sup>21</sup> allow more accurate identification of individual cells in sections than do routine preparations.

### Touch Preparations

The freshly obtained biopsy specimen can be picked up with forceps and gently applied to a clean glass slide by repeatedly touching the slide with the specimen. Touch preparations yield more certain identification of individual cells than do biopsy sections and are less likely than marrow smears to disrupt syncytial masses or compact clusters of cells.

## Examination of Bone Marrow Specimens

Information that can be derived routinely from examination of the marrow includes estimation of cellularity, detailed cellular structure, estimation of iron stores, and determination of the presence or absence of tumor cells, storage cells, and granulomas. Normal values are presented and indications for aspiration and/or biopsy will be discussed here, but details of abnormal findings in specific diseases will be given in later chapters.

### Estimation of Cellularity

Absolute counts of nucleated cells from marrow aspirates<sup>174</sup> are of little value because wide fluctuations in cell concentration are produced by aspiration of variable amounts of blood with marrow. Accurate techniques are available for estimating total nucleated cells in the bones of small animals,<sup>17</sup> but

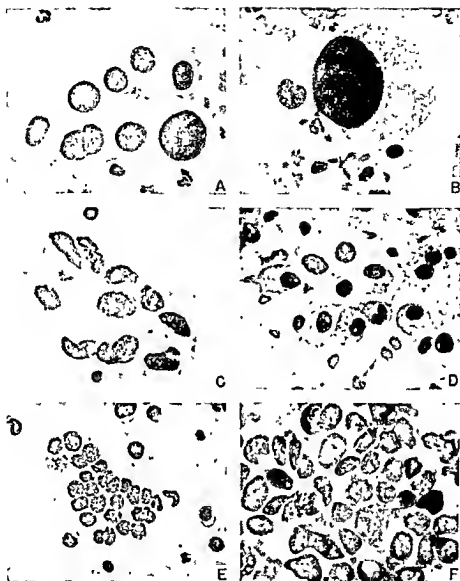


Fig 2-14 Tumor cells in bone marrow smears. A and B, mucus-producing cells from a patient with bronchogenic carcinoma. C, mucoid carcinoma of the colon. D, hypernephroma. E and F, small and large cell metastases from neuroblastomas. All  $\times 380$ .

these are not applicable to man. Examining smears of aspirated marrow gives little information concerning total cellularity of the marrow. One can state that the *smear* is rich in cells or relatively acellular, but to draw inferences concerning *marrow* cellularity from smears is hazardous. Somewhat more reliable information concerning marrow cellularity is obtained by examining sections made from biopsy specimens. In such specimens, fat spaces and hematopoietically active

parenchyma normally exist in a ratio ranging from 1:1 to 2:1 in adults. Deviation beyond these limits suggests that the marrow is hypoplastic or hyperplastic with respect to hematopoiesis. However, it must be remembered that such a biopsy specimen represents but an infinitesimal fraction of the total marrow. There is evidence for fairly even distribution of the *proportion* of various cell types throughout the marrow (page 69), but whether total cellularity is evenly distrib-

uted throughout the marrow is less certain. Significant variation in cellularity in different portions of large sections from the iliac crest has been reported.<sup>91</sup> If total cellularity is an important concern in the diagnostic or therapeutic considerations, then one of the techniques for estimating total marrow mass (page 61) should be considered. The use of the myeloid to erythroid ratio (M:E) in estimating cellularity is discussed on page 71.

### Cells of the Normal Bone Marrow

Cellular identification is much more certain in properly prepared smears from aspirated marrow than in sections of biopsied marrow.

#### Megakaryocytes

Concentration should be estimated in smears of aspirated material and/or in sections of biopsied material by scanning the preparations under low-power magnification (for example, at 100 $\times$ ). In specimens rich in normal hematopoietic tissue, these giant cells (Plates VIII, XIV) should be found easily in each field. Low-power scans should also be carried out routinely for the purpose of detecting clusters of tumor cells or granulomas (Figs. 2-14 and 2-15 and Plate XXIV).

#### Marrow Differential Count

This is carried out by examining a minimum of 300 to 500 nucleated cells under oil-immersion magnification. Care must be taken to examine well-spreadout, well-stained areas in which few ruptured cells ("bare nuclei") are present.

Values for the percentage of various cells in marrow vary rather widely in reported series. This could be attributed to differing morphologic definitions of cells, variations in amount of contamination by blood leukocytes, the inclusion or exclusion of areas with many ruptured cells, and inclusion of sick patients with "normal blood" as sources of normals. Results of differential counts from smears prepared from marrow particles

obtained by sternal aspiration (page 63) from 12 healthy men are given in Table 2-1. These values are not presented as "exact" normals but as a guideline for what may be expected. They differ from those summarized by Osgood and Seaman<sup>156</sup> only in the slightly lower percentage of segmented neutrophils and a higher percentage of nucleated erythrocytes. Custer<sup>56</sup> reported a higher percentage of myelocytes and a much lower percentage of lymphocytes than are shown in Table 2-1.

Glaser and associates<sup>86</sup> and Sturgeon<sup>200</sup> as well as others<sup>63,80,192,214</sup> reported extensive studies of marrow aspirates from normal infants and children. These studies are summarized in Table 2-2. At birth there are few lymphocytes in marrow aspirates but by one week of life an increasing percentage of lymphocytes is noted. A third to more than half the marrow cells are lymphocytic during the remainder of the first year of life, but their percentage declines to approach adult levels by the age of four years. During the first week of life the percentage of erythrocytic precursors declines quite rapidly because of lack of erythropoietic stimulation (page 56). Thereafter the M:E ratio remains essentially the same throughout infancy and childhood and into adult life. There is a tendency for eosinophils to be increased in young children. Plasma cells are rarely observed in the marrow of newborn infants but appear during the first few months of life (Chapter 7).

In our series (Table 2-1) as well as in most studies which provide the necessary raw data, the distribution of the percentage of various cell types is gaussian except for plasma cells and perhaps for lymphocytes. The distribution of both of these cell types tends to be skewed toward high values. This suggests that lymphocytes and plasma cells are more unevenly distributed in the marrow than are other cells. This postulate tends to be borne out by examination of sections of biopsy specimens in which irregularly spaced lymphocytic nodules may be discerned<sup>193</sup> and in which plasma cells tend to be associated with blood vessels.

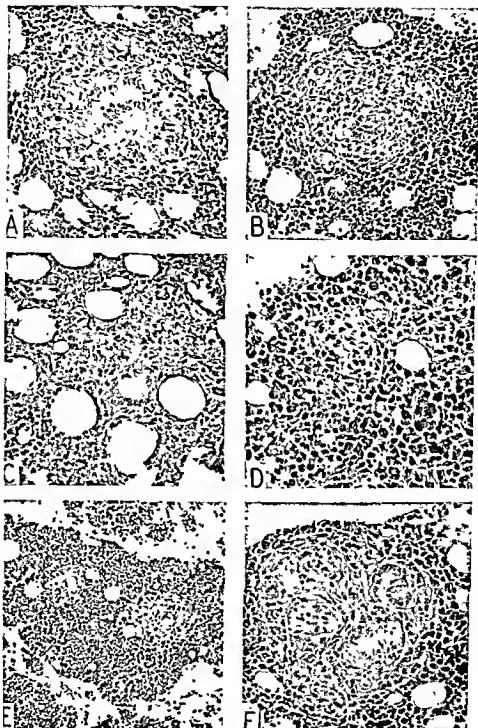


Fig 2-15. Granulomatous lesions in sternal bone marrow. A, Miliary tuberculosis ( $\times 205$ ), B, sarcoidosis ( $\times 225$ ), C, Hodgkin's disease ( $\times 285$ ), D, infectious mononucleosis ( $\times 350$ ), E, granulomatous hepatitis ( $\times 235$ ), F, acquired hemolytic anemia, history of undulant fever seven years before ( $\times 145$ ), hematoxylin and eosin stain (From Pease,<sup>168</sup> courtesy of author and Grune & Stratton)



Table 2-2. Changes in Differential Counts of Bone Marrow with Age

		Birth	1 Week	1 Week to 1 Year	1-4 Years	4-12 Years	Adult
Neutrophilic series	$\bar{x}\%$	54	65	37	50	52	57
	95% limits	31-77	21-79	22-52	32-68	35-69	39-79
Eosinophilic series	$\bar{x}\%$	3	3	3	6	3	3
	95% limits	1-5	1-5	1-5	2-10	1-5	1-5
Lymphocytes	$\bar{x}\%$	6	13	36	22	18	17
	95% limits	2-10	7-19	18-54	8-36	12-28	10-24
Erythrocytic	$\bar{x}\%$	34	15	17	19	21	20
	95% limits	18-50	5-25	7-27	11-27	11-31	10-30
M:E ratio	$\bar{x}$	1.6	4.3	2.2	2.6	2.5	2.6

The means ( $\bar{x}$ ) and 95% confidence limits in this table were calculated by combining data published by Osgood and Seaman<sup>154</sup> Sturgeon<sup>200</sup> Shapiro and Bassen<sup>192</sup> Veeneklaas and associates<sup>214</sup> and Olwany,<sup>43</sup> and the data in Table 2-1

### Normal, Nonhematopoietic Cells (Plate 1)

These include reticulum cells, osteoblasts, osteoclasts, and Schwann cells.

*Reticulum cells* range between 20 and 30  $\mu\text{m}$  in diameter (Plate XXIV, F). Their cytoplasm is pale, basophilic, and abundant, and may contain a few azurophilic granules. The nucleus is large, round, or oval and presents a pale-staining, fine, lace-like chromatin pattern and one or more round or oval nucleoli.

*Osteoblasts and osteoclasts* must be differentiated from the hematopoietic cells of the bone marrow since they may be mistaken for cells of the plasmacyte series and megakaryocytes, respectively, or even for tumor cells invading the marrow. *Osteoblasts* are oval cells, sometimes elongated, 25 to 50  $\mu\text{m}$  in diameter, with rather blurred outlines. The cytoplasm may take a light-blue or sometimes a dark-blue stain, may contain a few azurophilic granules, and occasionally is fenestrated. As a rule the nucleus lies eccentrically and is composed of clumped or trabeculated chromatin and distinct parachromatin. It contains from one to three nucleoli. *Osteoclasts* are giant, polyploid cells with indistinct cytoplasmic borders, their diameters often exceeding 100  $\mu\text{m}$ . The cytoplasm is cloudy and finely granular in the marginal portions

and may stain from weakly basophilic to strongly acidophilic. It may contain numerous azurophilic granulations of various sizes. The nuclei of these polyploid cells are scattered throughout the cell, usually not touching one another. The nuclear chromatin is dense, but one nucleolus is usually present in each nucleus. *Osteoblasts and osteoclasts* are seen most frequently and in largest numbers in fetal marrow. In disease, they have been observed especially frequently in association with metastatic malignant lesions, acute leukemia, myelofibrosis, and secondary osteoporosis.<sup>92</sup>

*Schwann cells* may be observed in sections from biopsy specimens and probably are related to the innervation of bone marrow. They have not been described in aspirated material.<sup>37</sup>

### Necrotic Marrow

Necrotic marrow may be obtained from patients with leukemia, lymphosarcoma, or other forms of tumor invading the marrow.<sup>2,33</sup> This is easily recognized in marrow sections<sup>2</sup>; in smears the marrow cells appear smudged and are surrounded by slightly acidophilic, granular masses.<sup>33</sup>

### Myeloid-to-Erythroid Ratio

This ratio is derived by relating the percentage of neutrophils and neutrophil pre-

cursors to the percentage of nucleated erythroid precursors. Whether eosinophils and basophils are or are not included in the "myeloid" percentage is usually of little consequence. We prefer to exclude these cells since their production appears to be controlled independently of the neutrophilic series (Chapter 6). Some hematologists prefer to exclude mature neutrophils from the ratio, an exclusion which significantly lowers the "normal limits."<sup>57</sup> The extremes of M:E ratios in our studies of the marrow of 12 normal men were 1.5:1 and 3.3:1, with a mean of 2.3:1 (Table 2-1). Inferences concerning the total number of myeloid or erythroid cells in the marrow can be made from

this ratio provided there is evidence that one of the systems is normal. For example, if blood neutrophil concentration and cell structure are normal and if the ratio of various neutrophil precursors in marrow to one another is normal, it can be assumed this is a normal system. In this circumstance an increase in the M:E ratio implies decreased nucleated red cells and decreased red cell production; a decreased M:E ratio implies the converse. However, if both systems are abnormal, no estimates of total production in either system are justified from the M:E ratio. Examples of the anticipated changes in M:E ratios in various diseases are shown in Table 2-3.

Table 2-3. Representative Differential Counts of Bone Marrow Obtained by Puncture

Types of Cells	Leukemia, Acute <sup>a,b</sup>	Leukemia, <sup>b</sup> Chronic Myelocytic	Leukemia <sup>b</sup> Chronic Lymphocytic	Multiple Myeloma <sup>c</sup>	Pernicious Anemia	Hemolytic Anemia	Iron Deficiency Anemia	Purpura Hemorrhagica <sup>d</sup>
Myeloblasts	50 0 95 0	4 0		0 5	0 8	0 8	0 5	
Promyelocytes		10 0	0 8	1 8	2 7	3 0	2 0	1.5
Myelocytes								
Neutrophilic		26 0	1 5	1 8	7 7	8 0	8 0	8 0
Eosinophilic		2 0	0 7		0 8	2 0	0 8	
Basophilic		0 4	0 2		0 3			
Metamyelocytes		22 0	8 0	3 3	14 5	18 0	15 0	15.3
Polymorphonuclear								
Neutrophilic		29 0	8 5	62 0	14 5	8 0	28 0	31 0
Eosinophilic		0 8	1 0	3 5	0 5	0 6	0 2	0 5
Basophilic		0 4	3 0	1 2	0 2			0 2
Lymphocytes		1 4	60 0	13 0	9 5	10 0	1 0	2 5
Plasma cells				4 5*	0 2	0 4	0 7	0 8
Monocytes		0 2		0 2	0 3			
Reticulum cells		1 2	1 5	1 0	2 0	2 6	0 8	
Mitotic figures		0 2	0 3		2 7	1 0		
Abnormal cells								
Megakaryocytes								0.2 <sup>d</sup>
Megablasts					40 0			
Pronormoblasts			0 2			5 0		4 0
Normoblasts		2 4	14 3	9 0	3 0	43 0	40 0	36 0
Myel eryth (M:E) ratio		40:1	15:1	8:1	1:15	1:1	14:1	15:1

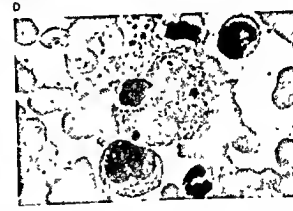
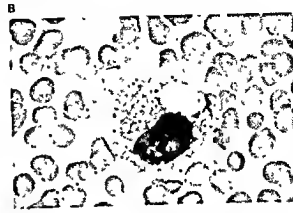
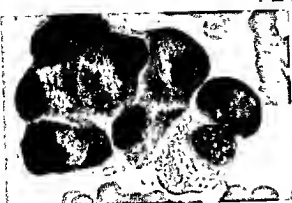
<sup>a</sup>The immature forms are listed in the table as myeloblasts merely as a matter of convenience. In acute lymphoblastic leukemia the cells are lymphoblasts, not myeloblasts. Often it is difficult to distinguish the various immature leukocytic cells seen in acute leukemia. The essential point is the great preponderance of very young forms.

<sup>b</sup>The bone marrow picture in *aleukemic leukemia* is similar to that of leukemia of the various types, whether or not changes can be demonstrated in the blood.

<sup>c</sup>The characteristic cells in multiple myeloma differ somewhat from typical plasma cells in that the nuclear chromatin is relatively fine and the wheel spoke arrangement of the chromatin is not present, the cytoplasm is basophilic and bright blue, not blue-green as in the plasma cell. A perinuclear clear zone is unusual.

<sup>d</sup>Although the number of megakaryocytes may not appear to be increased in typical purpura hemorrhagica the majority (64% in the patient cited) have no platelets about them and most of the remainder (32%) have very few.

# PLATE II



Giant cells found in the bone marrow (photomicrographs, Wright's stain,  $\times 1000$ ). A, Osteoblasts. B, multinucleated osteoclast; C, D, E, macrophages. F, "sea-blue" macrophage from a patient with chronic myelocytic leukemia. G, Gaucher cell, H, Niemann-Pick cell. Contrast these cells with the megakaryocytes shown in Plate VIII

## Hemosiderin in Bone Marrow

Examination of the bone marrow for iron is valuable in determining the adequacy of body iron stores. Several techniques are available. Particles of marrow, smeared on coverglasses, may be examined without staining.<sup>173</sup> After locating marrow tissue under low-power magnification, the particle is examined under oil-immersion magnification. Hemosiderin appears as golden yellow, refractile granules ranging from a fraction of a micron ( $\mu\text{m}$ ) to several micra in size. Marrow hemosiderin is more easily discerned by staining smears from aspirates or sections from biopsies with Prussian blue.<sup>67</sup> Large granules or aggregates of blue-staining iron may be observed within reticuloendothelial cells or they may appear to be lying free between cells in aspirated material. Smaller granules can be seen within developing red cells (sideroblasts) in properly prepared smears of normal marrow.<sup>107</sup> The significance of the proportions of nucleated red cells which are sideroblasts as well as the significance of reduced or increased iron in marrow are discussed in Part III, Section 3.

## Indications for Marrow Aspiration

Marrow aspiration is at all times interesting and often affords a more complete picture of the reaction of the hematopoietic tissue than can be gained from the blood sample alone.<sup>70,116,166</sup> However, it yields information of crucial importance in a limited number of conditions.

### Anemia

#### *Megaloblastic Anemias (Chapters 14 and 15)*

This type of anemia usually is evident from examination of the blood smear, but examination of aspirated marrow provides confirmation, if needed. Little, if any, diag-

nostic information is gained by examining the marrow of patients with nonmegaloblastic macrocytic anemias. An example of the expected findings in the marrow of patients with pernicious anemia is shown in Table 2-4.

#### *Hypochromic, Microcytic Anemias (Chapters 16, 17, and 18)*

Iron stains of aspirated material are useful when the diagnosis is not clearly established from the blood smear and from the iron and transferrin levels in serum. In patients with the rare sideroblastic anemias, the demonstration of iron granules "ringing" the nucleus of the nucleated red cells is essential to the diagnosis. In marrow smears treated with Wright's stain, a decreased M:E ratio often is observed in specimens from patients with iron deficiencies (Table 2-4).

#### *Hemolytic Anemias (Chapter 20)*

Marrow examination is of no diagnostic value except to confirm the presence of increased erythropoiesis in response to hemolysis (Table 2-4). Marrow aspiration can be useful in distinguishing "aplastic" from "hemolytic" crises as a cause of increasing anemia in patients with these diseases.

#### *Normochromic, Normocytic Anemias (Chapter 19)*

Marrow aspiration is essential to diagnosis of the rare state of "pure red cell aplasia." However, routine marrow examination is of minimal value in patients with other such anemias unless the condition is secondary to a neoplastic or granulomatous disease, in which instance a biopsy should be performed (see below).

#### *Neutopenia (Part V, Chapter 41)*

Marrow aspiration is useful in observing the relationship between various neutrophil precursors and determining the M:E ratio.

**Table 2-4. Conditions in Which Various Types of Reaction May be Observed, as Demonstrated by Bone Marrow Aspiration**

<i>M E ratio increased</i>	<i>Nonmyeloid cells increased</i>
Myeloid forms of leukemia	Other forms of leukemia
Majority of infections	Multiple myeloma
Leukemoid reaction	Metastases from carcinoma, etc
Decrease in nucleated red cells	Gaucher's disease, Niemann-Pick disease
	Aplastic anemia (usually relative increase only)
	Infectious mononucleosis
<i>M E ratio normal</i>	
Normal marrow	
Myelosclerosis	
Multiple myeloma, etc	
Aplastic anemia	
	<i>M E ratio decreased</i>
	Decrease in myeloid cells (agranulocytosis)
	Increase in erythroid cells due to either
<i>Normoblastic hyperplasia</i>	<i>or Megaloblastic hyperplasia</i>
Hemorrhagic anemias	Pernicious anemia
Iron deficiency anemia	Sprue, idiopathic steatorrhea, resection of small intestine (certain cases)
Hemolytic anemias	Tropical macrocytic anemia
Thalassemie	Nontropical nutritional macrocytic anemia
Cirrhosis of the liver	Macrocytic anemia with <i>Diphyllobothrium</i> infestation
Polycythemia vera	Megaloblastic anemia of infancy
Plumbism	Megaloblastic anemia of pregnancy
Anemia of chronic renal disease	Refractory megaloblastic anemia
	Achrestic anemia

### Leukemias (Part V, Chapters 46-49)

Marrow aspiration is a necessary diagnostic method in patients with acute leukemia who have few or no blast forms in the blood.

Marrow aspiration is of no diagnostic value in patients with the chronic leukemias whose blood is diagnostic except as a source of cells for cytogenetic search for the Philadelphia chromosome.

Examples of marrow findings in leukemia patients are given in Table 2-4.

### Thrombocytopenia (Chapter 34)

Marrow aspiration is useful in estimating whether megakaryocytes are increased, normal, or decreased. In the usual patient with idiopathic thrombocytopenic purpura, they are increased (Table 2-4).

### Immunoglobulin Disorders

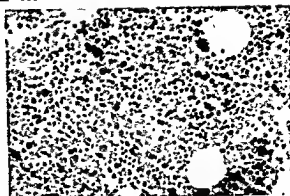
(Part V, Chapters 44, 45, 52, and 53)

Marrow aspiration is useful in demonstrating increased and sometimes abnormal-appearing plasma cells (Table 2-4) and lymphocytes in patients with immunoglobulin-producing tumors as well as in demonstrating absent or decreased plasma cells in those with the hypogammaglobulinemic syndromes.

### Miscellaneous Conditions

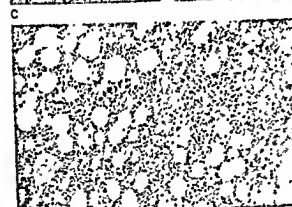
Certain diseases in which the diagnosis can be made from marrow, but not from blood, are Gaucher's disease (Plate II, G), Niemann-Pick disease (Plate II, H), and kala azar. Sometimes malarial parasites may be found by marrow aspiration when they cannot be demonstrated in blood.<sup>101</sup>

# PLATE III



A

B



**Bone marrow biopsies in different conditions (Giemsa stain)** A. Normal bone marrow ( $\times 100$ ) B. erythroid hyperplasia in a patient with hereditary spherocytosis ( $\times 400$ ) C. aplastic marrow of a patient irradiated for pelvic carcinoma ( $\times 100$ ) D. myelofibrosis ( $\times 100$ ) E. F. Hodgkin's disease (E.  $\times 100$ , F.  $\times 400$ ) G. Reed Sternberg-like cell ( $\times 1000$ ) H. Poorly differentiated lymphocytic lymphoma ( $\times 100$ ) (Courtesy of Dr Robert E. Lee)

Conditions in which marrow examination is of little or no diagnostic value include polycythemia (Chapter 30) and infectious mononucleosis (Chapter 43).

## Indications for Bone Marrow Biopsy

Marrow aspiration entails less discomfort and expense for the patient than does marrow biopsy. Consequently, biopsy should not be performed unless information additional to that obtained from aspiration is anticipated. Even if biopsy is carried out, a smear of the marrow should be made from a drop from the needle because this will be helpful in morphologic examination. In general, biopsy is indicated under the following circumstances:

1. Repeated failure to obtain adequate material by aspiration.

2. Evaluation of pancytopenia or bicytopenia. The evaluation of total cellularity afforded by biopsy (page 68) usually justifies its routine use when more than one type of blood cell is reduced. If only one cell line is reduced, similar information often can be gained from the M:E ratio (page 71).

3. With any blood changes raising the question of myelofibrosis (Chapter 57). In this circumstance, biopsy is required for confirmation since fibrosis cannot be demonstrated with certainty on smears from aspirations.

4. Any circumstance in which the question of metastatic tumor, lymphoma, or granulomatous disease of marrow can be raised. Tumor cells and even granulomas may be observed in aspirated material, but they are more easily found in sections from biopsy specimens.<sup>217</sup> Furthermore, since the *in situ* architecture of the marrow is better preserved in biopsied than in aspirated material, the identification of the lesion is aided by biopsy. Tumor cells, metastatic from carcinoma of the prostate,<sup>182</sup> breast, lung, kidney, and other tissues,<sup>67</sup> as well as in neuroblastoma<sup>79</sup> and rhabdomyosarcoma,<sup>61</sup> have been demon-

strated, even by marrow aspiration.<sup>147,208</sup> They are often found in groups or clumps (Fig. 2-14 and Plate XXIV). The cells usually are large, with vesiculated nuclei and scanty cytoplasm. The cytoplasm may be basophilic and mitotic figures may be found. One or more prominent nucleoli frequently are visible.

In sections of bone marrow it has been possible to demonstrate lesions of brucellosis,<sup>201</sup> miliary tuberculosis,<sup>187</sup> histoplasmosis,<sup>166</sup> and sarcoidosis,<sup>19</sup> as well as Hodgkin's disease<sup>67</sup> (Fig. 2-15).

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## *Morphology, Intrinsic Metabolism, Function, Laboratory Evaluation*

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Normal Development of the Erythrocyte

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### **Discovery of the Erythrocyte and Early Studies**

"Small round globules" were described in human blood by the Dutch microscopist, Leeuwenhoek, in 1673, but the "ruddy glob-

ules" were probably first observed by Swammerdam 15 years earlier. Malpighi (1665) mistook them for fat globules "looking like a rosary of red coral." Leeuwenhoek made a thorough study of these red bodies, and attributed the color of blood to them.

One hundred years later, William Hewson recognized that these particles are really flat discs rather than globules and suggested that they "must be of great use" in the body economy. The presence of iron in blood was demonstrated by Menghini in 1747, and Funke isolated hemoglobin in crystalline form in 1851. It was not, however, until 1867 that Hoppe-Seyler demonstrated that hemoglobin has the property of readily taking up and discharging oxygen. It was then that the functional significance of hemoglobin and of the particles in which it is carried became clear.

During the latter half of the 19th century, many studies of the erythrocyte were made. Vierordt (1852) and Welcker (1854) made the first blood counts. Their laborious technique was considerably improved by the invention of the counting chamber and diluting pipet. At the same time, attention was given to methods for measuring the coloring matter of the blood. Vierordt devised a spectroscopic method and Welcker described a colorimetric method. In the first monograph on hemoglobin, Preyer, in 1871, referred to several spectroscopic, chemical, and colorimetric methods.<sup>15</sup>

Neumann, in 1868, demonstrated that the red corpuscles are formed in bone marrow, arising there from colorless, nucleated elements.<sup>25</sup> With the introduction of the anilin dyes which followed Ehrlich's studies of 1877 and later, the morphologic study of the blood and tissues received attention. At the same time, interest was aroused in the variations in the size and hemoglobin content of erythrocytes in anemia and in methods for their measurement. These will be described later.

## Normal Development of the Erythrocyte

### The Erythron

Blood cells differ from the great majority of body tissues in that the mature, functioning cells are physically separated from their precursors. With respect to erythrocytes, it is useful to conceive of the precursor and mature cells as components of a single, though discontinuous, organ known as the "erythron."<sup>3</sup> This concept emphasizes the functional unity of the red corpuscles and their precursors. The erythron has been compared to the skin which evolves through layers of cells of increasing maturity, to a layer of essentially inert but functionally important cells, those of the corneum.<sup>41</sup> In the same way

one may think of the erythron with its primitive normoblasts giving rise eventually, after a series of changes, to the non-nucleated, highly specialized red corpuscles. The interstitial tissue of the erythron is represented by the plasma and by the fat and reticulum of the bone marrow. When considered as a whole, the erythron is an organ much larger than the liver (Fig. 3-1). The concept of the erythron as an organ unit contributes also to the understanding of pathologic changes in red cells (Chapter 13, page 529).

### Morphologic Aspects of Erythropoiesis

#### Nucleated Erythrocyte Precursors

*Erythroblast* was a term used by Ehrlich to refer to all forms of nucleated red cells, pathologic as well as normal. He classified erythroblasts into two main categories: a normal series, the *normoblasts*, and a pathologic series, the *megaloblasts*. The latter he had observed in pernicious anemia during relapse, as well as in early embryonic blood. Certain workers, however, notably Sabin and her school, used the term "megaloblast" to refer to a large and primitive, but normal, erythrocyte precursor. The term "megaloblast" will be used in this book in the pathologic sense given it by Ehrlich and followed by Naegeli, Ferrata, Downey, Jones, and others.<sup>25</sup> These

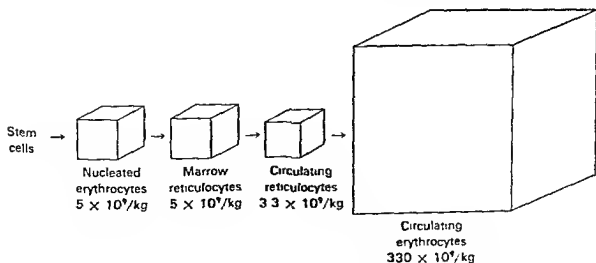


Fig. 3-1. Scale model of the erythron.<sup>8</sup> The relative proportions of each of the components are shown. The numbers below each box indicate the average number of cells per kilogram of body weight.

abnormal cells will be described and discussed in Chapter 14.

The existence of primitive hematopoietic stem cells of both pluripotential and unipotential varieties has been discussed in Chapter 2 (page 49). However, such cells have not been identified morphologically. The least mature, recognizable erythrocyte precursor cell is known as the *pronormoblast*. Cells characteristic of subsequent stages of maturation are termed *basophilic normoblasts*, *polychromatophilic normoblasts*, *orthochromatic normoblasts*, *reticulocytes*, and *mature erythrocytes*. A variety of synonymous terms have been applied by various authors (these will be given, in parentheses, with the descriptions of the stages which follow). The morphologic characteristics of each of these normal stages of erythrocyte maturation have been defined chiefly on the basis of observations with light microscopy. These characteristics will be described in the ensuing paragraphs, a description of additional findings on electron microscopy will follow (page 84).

The pronormoblast (proerythroblast of Ferrata, lymphoid hemoblast of Pappenheim, "rubriblast,"<sup>34</sup> "prorubricyte"<sup>34</sup>) is a round or oval cell of moderate to large size (14 to 19  $\mu$ m diameter). It possesses a relatively large nucleus and a rim of basophilic cytoplasm (Plate IV, B, Plate IX, A). The nucleus of the youngest cells in this group may differ but little from that of the myeloblast. Nucleoli are present and may be prominent. There is a very thin or delicate nuclear membrane. At this stage no hemoglobin can be made out in the cell, consequently its classification as a member of the red cell series is difficult. As compared with that of myeloblasts and lymphoblasts, the cytoplasm has a tendency to be more homogeneous and condensed and may appear granular. A small, pale area may be found in the cytoplasm, possibly corresponding to the Golgi apparatus.<sup>37</sup> The nuclear chromatin is somewhat more coarse than that in myeloblasts or lymphoblasts. In supravital stained preparations a number of rod-shaped mitochondria are seen in the cytoplasm.

The basophilic normoblast (basophilic

erythroblast of Ferrata, early erythroblast of Sabin, "basophilic rubricyte"<sup>34</sup>) is similar to the pronormoblast except that the nucleoli are no longer visible and the cell usually is somewhat smaller (12 to 17  $\mu$ m diameter) (Fig. 3-2, 3a; Plate IV, B; Plate IX, B). The chromatin has the appearance of coarse, granular material, and there is thus little resemblance to the myeloblast. The nuclear structure may assume a wheel-spoke arrangement ("Radkern"), and there tends to be a sharp contrast between chromatin and parachromatin. The cytoplasm is deeply basophilic, even more so than in the pronormoblast. This color is a manifestation of the presence of ribonucleic acid (RNA). The color changes during subsequent stages reflect the appearance of acidophilic hemoglobin and the disappearance of RNA.

The first faint blush of hemoglobin, as indicated by one or more pink areas near the nucleus in dry fixed preparations, introduces the next stage which is called the polychromatophilic normoblast (late erythroblast of Sabin, polychromatophilic rubricyte<sup>34</sup>) (Fig. 3-2, 3b; Plate IV, F; Plate IX, C, D). Increasing condensation of nuclear chromatin is observed during this stage. Irregular lumps of chromatin are formed, which may stain very deeply. Nucleoli are not visible. The nucleus is smaller (7 to 9  $\mu$ m) as is the cell as a whole (12 to 15  $\mu$ m). With supravital stains, the maximum number of mitochondria will be found in the early phases of this stage, but, as hemoglobin becomes more plentiful, mitochondria decrease in number.

When the cytoplasm possesses almost its full complement of hemoglobin, the cell is termed an orthochromatic (acidophilic) normoblast (Fig. 3-2, 3c; Plate IX, E), a designation meant to imply that the cytoplasmic hue is similar to that of mature erythrocytes. Strictly speaking, normoblasts are rarely fully orthochromatic, but this term is convenient in distinguishing the more acidophilic from the distinctly polychromatophilic stage. The orthochromatic normoblast is the smallest of the nucleated erythrocyte precursors (8 to 12  $\mu$ m in diameter).

In this final stage, the nucleus undergoes

# PLATE IV



*Megaloblasts and normoblasts, from bone marrow (Wright's stain,  $\times 1220$ ) A, C, E and G are from the bone marrow of patients with pernicious anemia in relapse and show various stages in the development of these cells. In the top row, a basophilic megaloblast (A) is contrasted with a basophilic pronormoblast (B) from a patient with idiopathic refractory anemia. The basophilic and polychromatic normoblasts in D and F are contrasted with megaloblasts (C, E, and G) at approximately corresponding stages of development.*

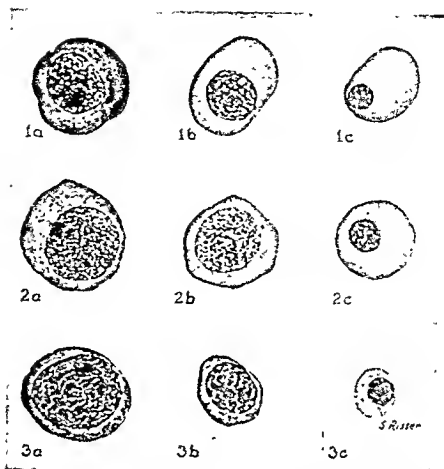


Fig. 3-2 Nucleated red cells from the rat yolk sac (1a b c) bone marrow of a patient with pernicious anemia (2a b c) and embryonic rabbit liver (3a b, c). Series 3 represents basophilic (a) polychromatophilic (b) and orthochromatic (c) normoblasts. Series 2 shows megaloblasts in the same order. Series 1 illustrates the appearance of the primitive erythroblasts found in the yolk sac. Dry imprint May-Grünwald-Giemsa stain prepared by Dr. A. Kirschbaum  $\times 1400$  (From Jones,<sup>21</sup> in *Downton's Handbook of Hematology*, courtesy of the author and Paul B. Hoeber, Inc.)

pyknotic degeneration, the chromatin becomes greatly condensed, and the nucleus shrinks. The nucleus may appear to be an almost homogeneous mass. It may assume various bizarre forms such as buds, rosettes, clover leaves, or double spheres, or only a faint ring may remain. That the changing pattern of the nuclear chromatin is not an artifact produced by fixation has been shown by studies with the electron microscope.<sup>29</sup> Distortions of this process have been described.<sup>16</sup> Finally the nucleus is lost.<sup>5</sup>

The proportions of various nucleated red cells in normal bone marrow are given in Chapter 2 (page 62).

### Reticulocytes

After the nucleus has been lost, certain cytoplasmic organelles, such as ribosomes, mitochondria, and the Golgi complex, persist for a short time. Methyl alcohol or similar fixative agents used in staining cause a uniform precipitation of the ribosomal RNA, a basophilic substance, and the intensity of the basophilic staining is roughly proportional to the amount of RNA present. After staining, such cells may appear uniformly blue or gray (*diffuse basophilia*), or various basophilic shades may be intermingled with pink-staining portions (*polychromatophilia* or



*polychromasia*). The effects of overstaining, which affects all the red corpuscles in the smear, must be distinguished from true basophilia which is found only in a small proportion of the cells.

*Punctate basophilia or basophilic stippling* is the term applied when there are bluish or bluish-black granules in red corpuscles stained by one of the Romanowsky methods. The granules may be fine or coarse, are usually uniform and round but may be angular, and have been seen in the unstained condition as well as by darkfield illumination.<sup>26</sup> Staining by special supravital methods demonstrates a third type of basophilia, the "substantia granulo-filamentosa" (Cesaris-Demel). These "skein cells" were first observed by Ehrlich and their significance was appreciated by Theobald Smith, but only much later did *reticulocytes*, as they came to be called, assume a position of importance in hematologic technology.

The *relationship of diffuse basophilia, stippling, and reticulocytes* has long intrigued hematologists and a close but imperfect parallelism between these three morphologic entities has been recognized. It can now be accepted that they are the result of different manipulations of the red cell in preparation for microscopy. Rapid fixation or strong fixing agents produce a high proportion of polychromatophilic cells and few stippled cells, whereas prolonged staining has the opposite effect.<sup>7</sup> That the basophilic material which is seen as "stippling" is composed wholly or in part of RNA is indicated by ultraviolet light absorption and by the fact that it disappears on treatment with ribonuclease.<sup>9</sup> By electron microscopy it can be seen in the form of ribosomal aggregates.<sup>20</sup> Non-heme iron and mitochondria may be associated with the aggregates, but can be differentiated.

In contrast to stippling, the "reticulum" of reticulocytes cannot be seen, even by dark-ground illumination or by phase contrast microscopy unless stained. However, examination by ultraviolet light with the wavelength that is absorbed by nucleic acids<sup>2</sup> and observation of the effect of ribonuclease on

the basophilic material<sup>9</sup> indicate that the reticulum, like the stippled material, also is composed in whole or in part of RNA. The precipitation of ribosomes by cresyl blue produces the reticular network visible by light microscopy.

In reticulocytes, the reticulum may appear as a narrow band traversing the cell, it may be evenly distributed throughout the cell, or it may be so densely packed as to give the appearance of a nucleus. Generally speaking, the amount of reticulum in reticulocytes decreases as the cells mature, and in "old" reticulocytes only a few granules or scattered threads may be found.<sup>17</sup> The shape and density of the network also depend, however, on a number of physical factors. Thus, the stronger the concentration of the dye, the larger and less broken up is the reticulum.<sup>7</sup> Drying of the film tends to produce a fine reticulum. Heating tends to destroy the reticulum, only rods and granules being demonstrable. A change in the pH of the staining mixture towards the acid side results in a finely granular reticulum, whereas treatment with dilute alkali produces a stippled form.<sup>28</sup> Substances such as glucose and sodium salts inhibit the staining of reticulocytes.<sup>18</sup> Crenation of red corpuscles is said to obstruct the passage of dye into the cells.<sup>7</sup>

Reticulocytes are larger than fully matured red corpuscles, perhaps 20% greater in volume.<sup>23</sup>

### *Electron Microscopic Findings<sup>22,29</sup>* (Figs. 3-3, 3-4 & 3-5)

Within hours after differentiation of the stem cell into the pronormoblast, *ferritin* can be found in the cytoplasm. On electron microscopy, this large, iron-containing protein has the characteristic appearance of a tetrad, making positive identification possible. Ferritin may appear as isolated molecules within the cytoplasm, or it may be found in pinocytic vesicles or in larger structures (often surrounded by a membrane) which have been called *siderosomes*. The sources and metabolic fate of ferritin will be discussed in Chapter 4 (page 162). Its morphologic importance is

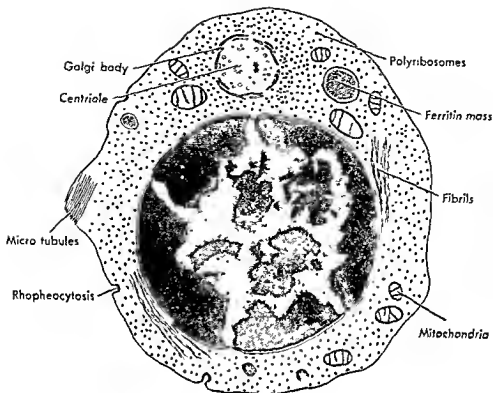


Fig. 3-3. Schematic diagram of the ultrastructure of the normoblast as visualized by electron microscopy (Courtesy of Dr. Marcel Bessis)

that it constitutes an important criterion in identifying a given cell as an erythrocyte precursor.

The cytoplasm of erythrocyte precursors contains *ribosomes*, but for the most part these remain free within the cytoplasm rather than being part of a well-defined endoplasmic reticulum. In cells which are actively synthesizing hemoglobin, the ribosomes are found in units known as polyribosomes; these consist of two to eight individual ribosomes joined together by a strand of messenger RNA. Ribosomes reach their maximum number during the basophilic normoblast stage and gradually disappear as the cell matures. They persist for several days following denudation and are the principal constituent of the "reticulum" demonstrable in reticulocytes by supravital staining procedures (page 31).

In erythroid cells, *mitochondria* are round or oval in shape, and the cristae are less distinct than in other cell lines. They are most numerous in the earlier stages of maturation, but some are found in reticulocytes as well

and may aggregate with ribosomes in the "reticulum."

Many small *vesicles* about 50 nm in diameter are seen throughout the cytoplasm. They have a single membrane with an indistinct inner layer, and they sometimes contain ferritin particles.<sup>37</sup> These vesicles are thought to arise by a process termed "pinocytosis" or "rhopheocytosis" (Fig. 3-3), whereby macromolecular substances are brought into the cell. The vesicle is formed from an invagination of the cell membrane, followed by closure to form a vacuole which later separates from the membrane.

Other cytoplasmic structures found in the young normoblast include a Golgi apparatus and occasional, randomly oriented *microtubules*. The latter are of unknown function, but may represent remnants of the marginal band, a cytoskeletal structure characteristic of erythrocytes of lower species.<sup>13</sup> Alternatively, they may be remnants of mitotic spindles.<sup>37</sup>

The *nucleus* of the pronormoblast is somewhat irregular in shape in contrast to the



**Fig 3-4** Basophilic normoblast viewed by electron microscopy. Compare with diagram of the ultrastructure of the erythroblast shown in Figure 3-3. Mitochondria, microtubules, fibrils, and polyribosomes are easily seen. (Courtesy of Dr. Marcel Bessis.)

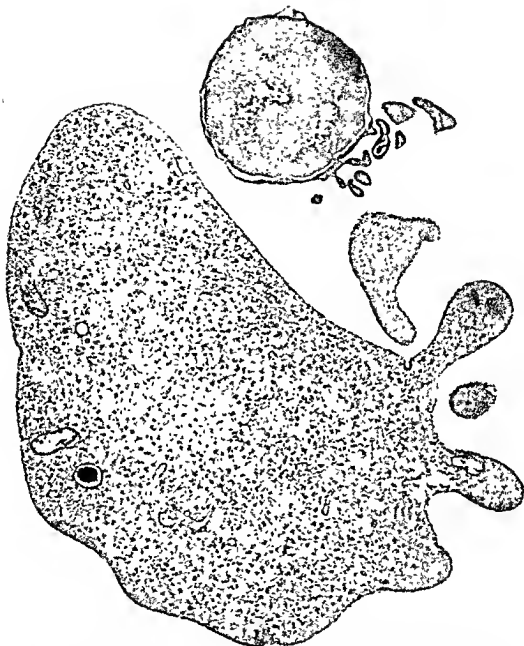
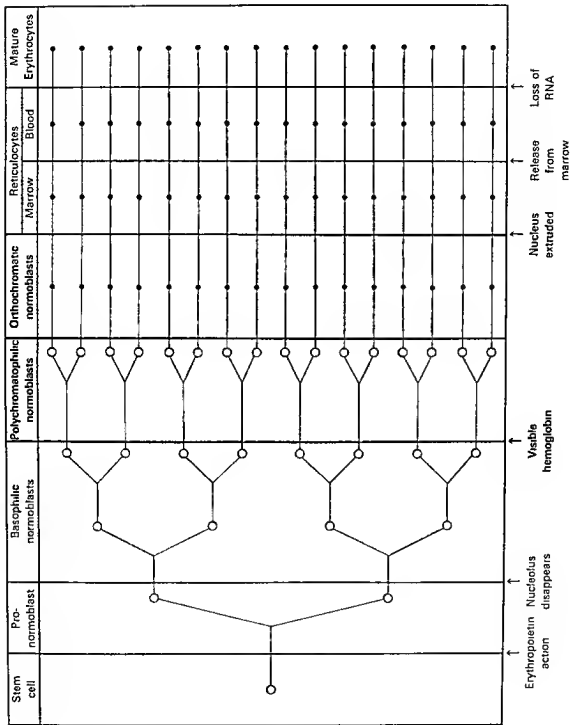


Fig. 3-5 A reticulocyte as viewed by electron microscopy is shown in the lower portion of this figure. Polynibosomes, mitochondria, and a large ferritin mass are easily seen. The irregular margin of the cell reflects the cellular movement that takes place at this stage.

A nucleus expelled from a normoblast is shown above the reticulocyte. A crown of cellular cytoplasm surrounds it. (Courtesy of Dr. Marcel Bessis.)

more nearly spherical form found in more mature cells. The nucleolus is large and well developed in the pronormoblast, but with increasing maturity it diminishes rapidly in both size and activity. In the basophilic normoblast, only traces of nucleolar material can be detected. Many nuclear pores connect

the nucleoplasm with the cytoplasm, and these pores decrease in number as the cell matures.<sup>57</sup> The nuclear chromatin appears homogeneous in pronormoblasts, but condensation (formation of heterochromatin) begins early in the basophilic normoblast stage. Condensation appears to begin near the



nuclear membrane and to extend thereafter toward the center in irregular radial lines. Finally, in the orthochromatic normoblast, the nucleus is nearly all heterochromatin.

### Proliferation and Maturation of the Erythron

Within the erythron, cellular maturation and proliferation proceed simultaneously. All morphologically identifiable erythrocyte precursors are destined to mature; thus, they are incapable of self-maintenance. Maintenance of the erythron at a given size and its expansion on demand are functions of the stem cell compartment (Chapter 2), and are under hormonal control (Chapter 4, page 180).

Details of the proliferation scheme within the erythron are incompletely understood since they must be based on certain assumptions. For example, the calculation of the time a cell spends in a given morphologic stage (the compartment transit time or CTT) depends on whether the products of mitosis are assumed to be recognizably more mature than the mother cell (ie, "heteromorphogenic" division) or whether the daughter cells are indistinguishable from the mother cell ("homomorphogenic" division).<sup>24</sup> Depending on which of these two types of division is assumed, the CTT for basophilic normoblasts may be as short as 12.4 hours or as long as 95 hours and for polychromatophilic normoblasts, 8.8 to 37.5 hours. The CTT for pronormoblasts is about 30 hours and for orthochromic normoblasts, 19 hours.<sup>24</sup> Thus, it takes from 70 to 180 hours for a pronormoblast to develop into a marrow reticulocyte. An additional two to three days elapse before release from marrow to blood.<sup>10</sup>

Probably three to five cell divisions occur during the maturation of erythroid precursors.<sup>31</sup> Thus, 8 to 32 mature red cells are derived from each pronormoblast. For illustrative purposes, the scheme outlined in

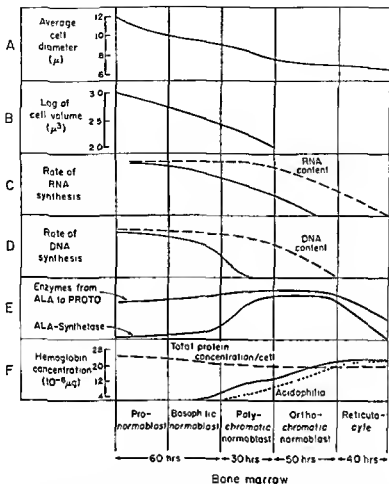
Figure 3-6 assumes that four divisions occur, two of these during the basophilic normoblast phase and one each at the end of the pronormoblast and polychromatophilic normoblast stages, respectively. Orthochromatic normoblasts cannot synthesize DNA, and, therefore, cannot divide.

Two events may decrease the theoretic yield of cells. One of these is the death of the cell prior to or shortly after its release from marrow ("*ineffective erythropoiesis*") (Chapter 13, page 550). The second is a skipped cell division, a phenomenon that results in a large, hemoglobin-poor cell (Chapter 14, page 567). Both of these events occur to only a limited extent in normal subjects, but may be greatly increased under pathologic circumstances.

Presumably, the differentiation of stem cell to pronormoblast is accomplished by the induction of certain genes, especially those necessary for hemoglobin synthesis, and by repression of others not required for erythrocyte function. The pronormoblast stage is dominated by the process of RNA synthesis, as is implied by the presence of the large and active nucleolus. Three species of RNA are formed—ribosomal, transfer (tRNA), and messenger (mRNA)—probably in relative proportions of approximately 80:15:5 respectively.<sup>12</sup> The synthesis of RNA declines as the cell matures (Fig. 3-7) and probably ceases toward the end of the basophilic stage.<sup>11</sup>

Proteins synthesized during the pronormoblast phase are mainly non-hemoglobin proteins. The first traces of hemoglobin may be detected in the basophilic normoblast stage, but hemoglobin is not apparent on light microscopy until the polychromatophilic stage. Here, the rate of hemoglobin synthesis reaches a maximum. Hemoglobin synthesis continues through the orthochromic stage and persists in the reticulocyte after denucleation. Mature red cells, being devoid of ribo-

Fig 3-6. Proliferation within the erythron as related to morphologic stage. Three to five divisions occur (four are shown here) between the pronormoblast stage and the reticulocyte. Thus there is a yield of 8 to 32 cells per pronormoblast. However, no cell at the orthochromatic stage or older is capable of mitosis. The principal events associated with the morphologic stages are indicated on the lower margin.



**Fig 3-7** Erythroid maturation alterations in cell size, rates of DNA and RNA synthesis, enzymes involved in heme synthesis, and hemoglobin concentration. Substances listed in the left hand column are represented by corresponding solid black lines. Unless specified, graphs represent relative values. ALA refers to  $\delta$ -aminolevulinic acid. PROTO refers to protoporphyrin. It will be noted that considerable protein synthesis takes place during the earliest phase. Following this the nucleus disappears but mitochondria remain. As the concentration of DNA decreases and the concentration of RNA starts to fall, hemoglobin begins to appear, increasing rapidly in amount. (From Granick and Levere,<sup>12</sup> courtesy of the authors and Grune & Stratton.)

somes, are unable to synthesize hemoglobin.

As previously noted, morphologic evidence of nuclear degeneration (heterochromatin formation) can be seen as early as the basophilic normoblast stage. By the orthochromatic stage, the nucleus is completely inactive, unable to synthesize either DNA or RNA. The factors leading to cessation of nuclear activity are not fully understood, but there is evidence that they may be related to intracellular hemoglobin concentration.<sup>35</sup>

Hemoglobin is found within the nucleus, possibly gaining entrance through pores in the nuclear membrane.<sup>27,35,39</sup> After reaching a critical concentration (possibly 20 g/dl)<sup>40</sup> nuclear hemoglobin may react with nucleohistones thereby bringing about chromosomal inactivation and nuclear condensation. According to this hypothesis, the number of cell divisions and the ultimate erythrocyte size are related to the rate of hemoglobin synthesis. For example, microcytic cells are

produced in iron deficiency because it takes longer to reach the critical hemoglobin concentration and the generation time is unaffected; hence, more cell divisions occur before nuclear inactivation, and the resulting cell is small. In contrast, the macrocytes observed when erythropoiesis is stimulated may be conceived as the end result of an erythropoietin-induced acceleration of hemoglobin synthesis which in turn leads to an earlier onset of nuclear degeneration and a reduced number of cell divisions. Also consistent with this hypothesis is the observation that the mean corpuscular hemoglobin concentration remains relatively constant in a variety of mammalian species, even though erythrocyte size varies greatly<sup>10</sup> (Fig. 3-18).

After the nucleus degenerates, it is extruded from the cell.<sup>1</sup> This process, which has been observed in living normoblasts by phase contrast microscopy,<sup>30</sup> is completed in 5 to 60 minutes. During the extrusion process, mitochondria and cytoplasmic vesicles accumulate near the nuclear border.<sup>33,37</sup> The role of these structures in nuclear extrusion is not entirely clear, but supravital staining with Janus green B, a mitochondrial toxin, inhibits denucleation.<sup>1</sup> The extruded nucleus carries with it a rim of cytoplasm (Fig. 3-5), including ribosomes, hemoglobin, and occasional mitochondria. Within the marrow, denucleation appears to occur as the erythroblast traverses the endothelial cell that forms the sinus wall.<sup>38</sup> The normoblast cytoplasm and small organelles (ribosomes and mitochondria) squeeze through endothelial, cytoplasmic pores 1 to 4  $\mu\text{m}$  in diameter, but the more rigid nucleus cannot conform to this pore size. The nucleus thus becomes caught and "pitted" from the cell. Soon after denucleation, the nucleus is engulfed by a macrophage.

Following denucleation, the cell remains within the marrow as a reticulocyte for several days. Factors controlling release into the circulation have been discussed in Chapter 2 (page 60).

After release, the reticulocyte may be sequestered for one to two days in the spleen.<sup>31</sup> Here, additional maturation may occur, and

the composition of membrane lipids may be altered.<sup>32</sup>

Reticulocytes are more adhesive than adult corpuscles and move about in currents at a much slower rate than do mature cells.<sup>7</sup> This adhesive property, like that described for more primitive cells,<sup>19</sup> may account for their tendency to remain within the bone marrow under normal conditions (page 60). They appear to have a coating of globulin,<sup>36</sup> at least part of which is transferrin.<sup>20</sup> Their specific gravity is lower than that of adult corpuscles<sup>22</sup> and they tend to collect in the upper portions of suspensions of corpuscles. They vary in their resistance to hypotonic solutions.<sup>6</sup> They have metabolic pathways that are lacking in mature red cells, including an intact tricarboxylic acid cycle.<sup>11</sup>

## The Mature Erythrocyte

The mature mammalian erythrocyte is one of the most highly specialized of cells. It is devoid of the usual cytoplasmic organelles such as nucleus, mitochondria, or ribosomes. Thus, it consists of little more than a membrane surrounding a solution of protein and electrolytes. More than 95% of the protein is hemoglobin. The remaining protein includes those enzymes required for energy production and for the maintenance of hemoglobin in a functional, reduced state.

### Shape and Dimensions

The resting shape of the normal human erythrocyte is that of a flattened, bilaterally indented structure, a shape often referred to as a "biconcave disc" (Fig. 3-8). In fixed, stained blood smears, only the flattened surfaces are observed; hence, the appearance is circular, with an area of central pallor corresponding to the indented areas (Fig. 1-7, page 27).

The normal mean diameter of red cells, measured after drying and staining, is variously given as 7.2 to 7.9  $\mu\text{m}$ .<sup>61,93,117</sup> Means as low as 6.9<sup>91</sup> and 6.5  $\mu\text{m}$ <sup>61</sup> have been reported in normal persons. In individual preparations, cells as small as 4.75  $\mu\text{m}$  and as large as 9.5  $\mu\text{m}$  may be found,<sup>91</sup> but generally the





Fig 3 8 The normal mature erythrocyte as visualized by the scanning electron microscope ( $\times 9800$ ) (Courtesy of Dr Wallace N Jensen)

greatest variation in the diameters of the cells is  $3.5\text{ }\mu\text{m}$ .<sup>61</sup> Diameters measured in wet films are  $0.8$  to  $1.4\text{ }\mu\text{m}$  greater than those just mentioned.<sup>51,115</sup> The normal mean thickness of red corpuscles has been reported to be  $2.14\text{ }\mu\text{m}$ ,<sup>94</sup>  $2.05\text{ }\mu\text{m}$ , or  $1.84\text{ }\mu\text{m}$ , and even  $1.64\text{ }\mu\text{m}$ .<sup>115,117</sup>

The mean volume of the normal human erythrocyte is about  $87\text{ fl}$  (range  $80$  to  $96$ )<sup>52,70,79,81,105,118</sup> as calculated from the ratio of the volume of packed red cells and the erythrocyte count (see page 116). Somewhat greater values, approximately  $108\text{ fl}$ , have been suggested from measurements

made on microscopy of hanging-drop preparations.<sup>54</sup>

The surface area of the erythrocyte<sup>54</sup> is about  $140\text{ }\mu\text{m}^2$ , considerably greater than that expected if volume were distributed in a sphere.

From cinematomicrographic observation of red cells within blood vessels, it is clear that their shape can be easily altered to facilitate passage through small vessels.<sup>104</sup> In such studies, the plane of the biconcave disc is found to be oriented in the direction of flow. The leading edge becomes pointed and the following edge blunted (Fig. 3-9); thus, the shape becomes similar, to that of a parachute or torpedo viewed from the side. When deformed in this way, the erythrocyte can pass through a vessel of about  $4\text{ }\mu\text{m}$  in maximum diameter. Because of the disc shape, alterations can be accomplished with relatively little change in surface area. If the erythrocyte were spherical in shape, considerable stretching of its membrane would be required to enable it to conform to small vessels.

The forces that cause the erythrocyte to maintain its resting, biconcave form are only partially understood. It has been suggested that the cell simply assumes the shape that utilizes the minimum energy of bending (least total curvature).<sup>63</sup> Others have calculated that the interplay of three distinct forces is required to maintain shape: a hydrostatic pressure difference across the membrane, a constant tension within the membrane, and

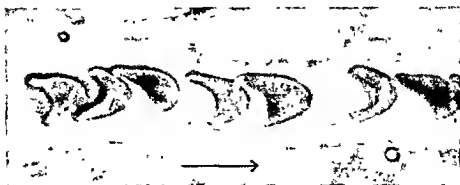


Fig 3 9. Human erythrocytes flowing through a vessel about  $7\text{ }\mu\text{m}$  in diameter. Direction of flow is indicated by the arrow (From Skalak and Branemark,<sup>104</sup> courtesy of the authors and Science)

a third force acting to indent the two sides.<sup>82</sup> It is possible that this apparent third force is the consequence of a variation in surface tension resulting from the peculiar distribution of membrane cholesterol.<sup>87</sup> However, observations with polarized light have indicated that there is an internal organization of molecules that contributes to shape.<sup>103</sup> One simple proposed model compares the erythrocyte to a partially deflated tennis ball, the equatorial region of which has been strengthened by an increased wall thickness or by fibrillar structures.<sup>92</sup> In such a model, the biconcave shape is stable.

### The Erythrocyte Membrane

The erythrocyte membrane is a dynamic component of the cell. It has the property of selective permeability, and it facilitates the

passage of cations against an ionic gradient. It also serves as the site of attachment for certain enzymes. By these means, it maintains the intracellular fluid and electrolyte environment within relatively narrow limits.

### Models of Membrane Structure

It is not possible to characterize the membrane completely in molecular terms. A variety of models of membrane structure have been proposed. These fall generally into two main categories: those based on the unit membrane, or lipid bilayer model originally proposed by Danielli<sup>88,89</sup> and modified by Robertson;<sup>90,97</sup> and those based on structural or functional subunits, a concept developed by Green and coworkers from studies of mitochondrial membranes.<sup>69</sup> The central feature of lipid bilayer models is an orderly arrangement of phospholipids into a sheet two molecules thick (Fig. 3-10A). The molecules are so oriented that the nonpolar groups of the two layers are directed toward one another, forming lipid-lipid interactions. The polar groups are directed outward and interact with protein on both the extracellular and intracellular surfaces.

It seems clear that this relatively homogeneous structure must be interrupted at some

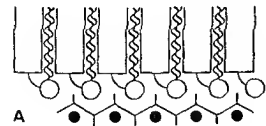
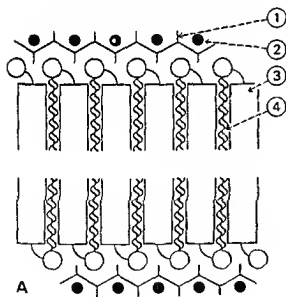


Fig 3-10. Models of membrane structure. A, Lipid bilayer membrane model. 1 Protein polypeptide chain, 2 mucopolysaccharide, 3 phospholipid, circles indicate the polar end. 4 cholesterol (From Fikrin and Wiley,<sup>84</sup> courtesy of the authors and Grune & Stratton.)

B, Subunit membrane model. Left, single subunit with hydrophilic regions at each end of a hydrophobic cylinder. Center, two-dimensional aggregate of subunits. Right, surface view (From Zahler,<sup>122</sup> courtesy of the author and Expenientia.)



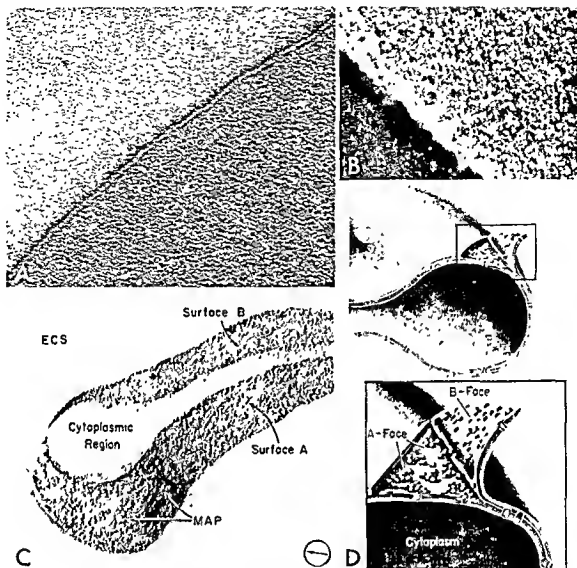


Fig 3-11. Ultrastructure of the erythrocyte membrane. A, Thin section, B, metal shadowed surface, C, freeze cleaved. Two surfaces are shown designated A and B, differing in the number of membrane-associated particles (MAP). D, Artist's conception of the surfaces exposed by freeze cleaving. (From Hoffman,<sup>74</sup> Robertson,<sup>77</sup> and Weinstein and McNutt<sup>114</sup> courtesy of the authors and American Journal of Medicine, Archive of Internal Medicine and Seminars in Hematology, respectively.)

points in order to provide for certain membrane functions. In these areas, the lipid bilayer may be replaced or modified to form a functionally specialized site. For example, it has been estimated that there may be 100 to 200 sites at which  $K^+$  transport occurs.<sup>73</sup> Such sites may occupy only a small proportion of the membrane area.<sup>106</sup>

Membrane models of the subunit type (Fig. 3-10B) are based on the assumed exis-

tence of one or a few classes of lipid-protein structural subunits, usually of macromolecular size. These are joined together to form the membrane, and may, in fact, be capable of self-assembly. Functional subunits are also proposed, consisting of all the elements necessary to carry out a given function.

Evidence for and against the two types of models has been summarized in several reviews.<sup>66,106,122</sup>

## Ultrastructure

In thin sections of erythrocyte membrane, fixed with either osmium tetroxide or potassium permanganate, three distinct layers are observed (Fig. 3-11). There are two electron-dense (osmophilic) layers approximately 2.5 nm (25Å) in thickness separated by an electron penetrable layer about 2.0 nm thick, for a total thickness of some 7.0 nm.<sup>50</sup> This appearance has often been cited in support of the lipid bilayer model, with the electron-dense areas representing either membrane protein layers or the polar ends of the phospholipids.<sup>97</sup> There is no agreement, however, on the molecular meaning of the three layers, since the reactions of osmium and permanganate with membrane components are not fully understood.

With air-dried, metal-shadowed red-cell membranes, features of the surface are made apparent.<sup>72</sup> In such preparations, plaques about 3.0 nm thick and 10 to 50 nm in diameter are observed, and these are distributed randomly over the surface (Fig. 3-11). These observations have been used to support the subunit model of membrane structure, with each of the plaques representing the morphologic equivalent of a lipid-protein subunit. However, a major criticism of such preparations is that it is not possible to distinguish structures that are part of the membrane from extraneous material on the membrane surface.

Still another technique used in electron microscopy of membranes is that of "freeze-cleaving."<sup>114</sup> Erythrocytes are frozen rapidly at  $-150^{\circ}\text{C}$  and cleaved with a razor blade. The cleavage plane follows pathways of least resistance, frequently exposing large areas of membrane. These surfaces are replicated with condensed carbon and platinum and the replicas are examined with the electron microscope. Two types of membrane surfaces are observed with this technique (Fig. 3-11). Both surfaces are characterized by the presence of particles approximately 10 nm in diameter. The two surfaces differ in that one has four to five times as many of these particles as the other (2600 to 3800/ $\mu\text{m}^2$  as com-

pared with 575 to 1400/ $\mu\text{m}^2$ ). Although interpretation of these observations remains controversial, the best evidence suggests that the cleavage plane splits the normal membrane into two halves (Fig. 3-11). If the lipid bilayer model is correct, the split may occur between the two lipid molecules; i.e., in the nonpolar region. Some of the observed particles can be shown to penetrate through the entire thickness of the membrane, suggesting that they may represent specialized functional sites.

## Chemical Composition of the Membrane

Much that is known about red cell membranes is derived from studies of the insoluble portion of the cell remaining after hemolysis. This material is called *stroma* (or, if the membrane remains intact after hemolysis, red cell "ghosts") and consists largely of components of the membrane.

It is difficult to prepare stroma completely free of hemoglobin. This observation led to the speculation that hemoglobin might be an integral part of the membrane. However, this hypothesis was doubted when it was found that labeled hemoglobin exchanged freely with stroma-bound hemoglobin.<sup>73</sup> Furthermore, with careful attention to pH and osmolarity, it has been possible to prepare stroma essentially free of hemoglobin.<sup>60,113</sup> About 230 to 300 mg of stroma can be extracted from 0.1 liter of erythrocytes. Such preparations contain 40 to 50% protein, 35 to 45% lipid, and 7 to 15% carbohydrate.<sup>51,60,121</sup> Earlier determinations reported a higher proportion of protein, probably because of a greater degree of hemoglobin contamination.

**LIPID COMPOSITION.** Of the three classes of membrane components the most detailed information available concerns lipids. Virtually all of the lipids in the mature erythrocyte are found in the membrane.<sup>60</sup> Qualitative and quantitative analyses have been performed in a number of laboratories and the data have been the subject of several reviews.<sup>55,107,111,112</sup> These results are summarized in Table 3-1. Values in children differ only slightly from those found in adults.<sup>88</sup>

Table 3-1. Lipids of the Normal Human Erythrocyte Membrane

Lipid	Molar Concentration <sup>101</sup>		Weight Concentration <sup>55</sup>	
	( $\mu$ mol/ $10^{10}$ cells)	% of total	(mg/ $10^{10}$ cells)	% of total
Neutral lipids				
Cholesterol	3.2		1.3 (1.1-1.4)*	
Free fatty acids	<0.1			
Total neutral lipid	3.2	43	1.3	29
Phospholipids				
Phosphatidylcholine (lecithin)	1.2		1.0	
Phosphatidylethanolamine (cephalin)	1.1		0.9	
Sphingomyelin	1.0		0.8	
Phosphatidylserine	0.6		0.4	
Lysolecithin	0.04		—	
Others	0.07		—	
Total phospholipids	4.0	54	3.1 (1.7-3.2)*	69
Glycolipids (globoside)	0.2	3	0.1	2
Total lipids	7.4	100	4.5 (3.9-5.2)*	100

\*Ranga<sup>111</sup> in parentheses

The neutral lipid of the erythrocyte consists almost entirely of free, non-esterified cholesterol.<sup>69</sup> Trace amounts of free fatty acids may also be found. The suggestion of the presence of cholesterol esters and triglycerides offered by earlier studies can probably be explained by the presence of contaminating traces of plasma lipoproteins.<sup>69</sup>

Most of the phospholipid of the erythrocyte membrane is accounted for by four classes of compounds: phosphatidylcholine (lecithin), phosphatidylethanolamine, sphingomyelin, and phosphatidylserine (Table 3-1). To each of these lipids are attached two fatty-acid side chains, except for sphingomyelin, which has but one. Trace amounts of other phospholipids containing only one fatty acid ("lysophospholipids"; eg lysolecithin) or in which a fatty acid has been replaced by a vinyl ether (plasmalogens) are found.

Of the fatty acids found in red cell phospholipids, about half are saturated and half are unsaturated (Table 3-2). These fatty acids

are not evenly distributed among the phospholipids. For example, lecithin contains most of the shorter-chained, saturated fatty acids, phosphatidylethanolamine is rich in unsaturated fatty acids; stearic acid predomi-

Table 3-2. Fatty Acids in Erythrocyte Phospholipids

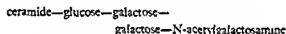
Fatty Acid	Molecular Designation*	%
Saturated		
Palmitic	16:0	24.5
Stearic	18:0	19.0
Others		3.1
Total saturated		46.6
Unsaturated		
Oleic	18:1	16.4
Linoleic	18:2	11.2
Arachidonic	20:4	15.1
Others		10.3
Total unsaturated		53.0

\*The first number indicates the number of carbons, the second, the number of double bonds

From Ways and Hanahan,<sup>112</sup> courtesy of the authors and Journal of Lipid Research

nates in phosphatidyl serine; and fatty acids of chain length longer than 20 are found, especially in sphingomyelin.<sup>55</sup> The fatty acid composition of the membrane influences its rigidity; saturated fatty acids, being less fluid, lend stiffness to the membrane.

The glycolipids (or glycosphingolipids) make up a small fraction of the total lipids of the erythrocyte membrane, but are, nevertheless, of unique biologic and pathologic importance.<sup>107</sup> These glycolipids resemble sphingomyelin in that the lipid base is a unit known as *ceramide*, consisting of sphingosine and a long-chain fatty acid. To the base is attached a variable number of hexose molecules. The predominant erythrocyte glycolipid, *globoside* (GL-4), contains four hexose units:



Globoside appears to be a component only of plasma membranes as distinguished from the membranes of intracellular organelles, such as mitochondria. In this respect, it differs from cholesterol and the phospholipids, which are widely distributed. Globoside is particularly characteristic of the red cell membrane, but has also been isolated from leukocytes and the kidney and may be present in plasma membranes of still other cells.

Normal human red cells also contain ceramide glycolipids with 1 glucose (GL-1), 1 glucose and 1 galactose (GL-2), and 1 glucose and 2 galactose molecules (GL-3) attached. The relative proportions of these are as follows: GL-4, 69%; GL-3, 12%; GL-2, 14%; and GL-1, 5%.<sup>107</sup> In addition, there are trace amounts of fucose-containing ceramide glycolipids. These are of interest because they have antigenic activity corresponding to the A, B, H, and Lewis blood groups. They may not be the exclusive bearers of these antigens, however, since glycoproteins with similar hexose arrangements have similar antigenic properties (page 100).

**LIPID TURNOVER AND ACQUISITION.**<sup>101</sup> The mature erythrocyte is unable to synthesize lipids *de novo*; therefore, any lipid loss must

be compensated for by renewal from pathways of interchange with the plasma (Fig. 3-12). Quantitatively, the most important of these pathways are the transfer of cholesterol and lecithin (phosphatidyl choline) from plasma lipoproteins to red cells (pathways 1 and 3). The rates of transfer are functions of the relative plasma and red cell levels of these lipids and are indirectly affected by the activity of the cholesterol esterifying enzyme in plasma, lecithin-cholesterol acyltransferase (LCAT).<sup>68</sup> This enzyme catalyzes the reaction in which a fatty acid in the 2 position of lecithin is transferred to free cholesterol, forming cholesterol ester and lysolecithin (Fig. 3-12, reaction 1A). Neither of the LCAT reaction products can enter the membrane.

The activity of LCAT probably accounts for the observation that cholesterol is lost from the membrane when erythrocytes are incubated in normal, glucose-containing plasma.<sup>57</sup> The cholesterol loss is accompanied by a decrease in red cell surface area and a spheroidal change in red cell shape. These abnormalities are reversible when cells are infused *in vivo*. Conversely, in patients with congenital LCAT deficiency, membrane cholesterol and lecithin are increased and the red cells are target-shaped.<sup>91</sup>

The exchange of cholesterol and lecithin between red cells and plasma also is affected by the plasma bile-salt concentration.<sup>56</sup> If erythrocytes are incubated in normal plasma to which bile salts have been added, the cells acquire cholesterol, and this change is accompanied by an increase in surface area and the formation of target cells. Although the mechanism of bile-salt action is not fully understood, at least two properties appear important: bile salts inhibit the LCAT reaction and, in addition, they bring about a shift in the distribution of free cholesterol between plasma and cell.

Phospholipids also may be added to the membrane by three other reactions. An albumin-bound lysophospholipid may be transferred to the membrane (Fig. 3-12, pathway 4) and acylated (reactions 5a and 6a) to form a complete phospholipid.<sup>101</sup> Of lesser quantitative importance is the transfer

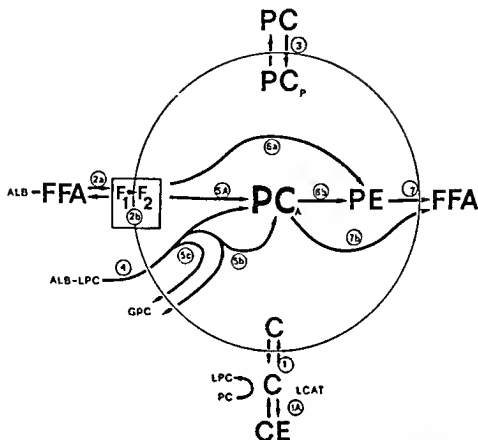


Fig 3-12 Pathways of lipid acquisition and turnover in the mature red cell membrane. C, Cholesterol; CE, cholesterol ester; PC, phosphatidylcholine (lecithin); LPC, lysophosphatidylcholine; PE, phosphatidylethanolamine; LPE, lysophosphatidylethanolamine; FFA, free fatty acid; Alb, albumin; GPC, glycylphosphorylcholine; LCAT, lecithin-cholesterol acyltransferase. *Reactions and pathways:* 1, Exchange of cholesterol with plasma lipoprotein; 1a, the LCAT reaction; 2a, transfer of FFA from albumin to membrane; 2b, penetration of FFA to a metabolically active site; 3, exchange of PC with plasma lipoprotein; 4, transfer of LPC from albumin to membrane; 5a,  $\text{LPC} + \text{FFA} \rightarrow \text{PC}$ ; 5b,  $2\text{LPC} \rightarrow \text{FFA} + \text{GPC}$ ; 5c,  $\text{LPC} \rightarrow \text{FFA} + \text{GPC}$ ; 6a,  $\text{LPE} + \text{FFA} \rightarrow \text{PE}$ ; 6b,  $\text{PC} + \text{LPE} \rightarrow \text{LPC} + \text{PE}$ ; 7,  $\text{PE} \rightarrow \text{LPE} + \text{FFA}$ ; 7b,  $\text{PC} \rightarrow \text{LPC} + \text{FFA}$ . (From Shohet,<sup>101</sup> courtesy of the author and New England Journal of Medicine.)

and conjugation of two lysophospholipid molecules to yield a phospholipid (reaction 5b) and glycyl-phosphorylcholine (GPC), which returns to the plasma.<sup>108</sup> Finally, some phosphatidyl ethanolamine (PE) is produced by transacylation of a fatty acid from lecithin to lyso-PE (reaction 6b). A congenital defect in the last reaction results in the formation of cells that possess increased membrane lecithin and decreased membrane PE. These changes are associated with the clinical picture of non-spherocytic hemolytic anemia<sup>102</sup> (Chapter 21).

The fatty acid composition, but not the

major relative proportions of the phospholipid classes, may be altered by dietary means.<sup>65</sup> With low-fat diets, linoleic acid decreases. With diets high in linoleic acid, red cell linoleic acid increases. These changes occur relatively slowly, over about a four- to six-week period.

Relatively little is known about the pathways of renewal of glycolipids. They appear to be synthesized in the bone marrow and incorporated into the membrane prior to release of the mature red cell.<sup>107</sup> It is possible that they remain with the cell throughout its life span. The enzymatic degradation of gly-

colipids occurs in macrophages by sequential cleavage of the hexose units. These reactions, and diseases which affect them, are discussed in Chapter 42.

**PROTEINS.**<sup>71</sup> The membrane proteins are not as well characterized as the lipids. Study of these proteins has been limited by the difficulty of extracting them from lipids and solubilizing them without destructive techniques. In early studies, the name "*elimum*" was applied to the insoluble membrane residue remaining after alkaline extraction and removal of lipids with ether.<sup>86</sup> It is now recognized that this material is a crude mixture of proteins, many of which are denatured.

Complete solubilization of membrane proteins has been accomplished by successive extraction with a chelating agent (EDTA), 0.8 M sodium chloride, ethanol-ether, and detergents (3% sodium dodecyl sulfate).<sup>98</sup> The detergent-soluble material has been further fractionated by gel-filtration. With these methods, seven to nine fractions of membrane proteins can be defined, and further heterogeneity can be detected by amino-acid end-group analysis in some of the fractions. These observations indicate that there are a minimum of 12 proteins (or polypeptide chains) in the membrane, and there may be many more than this minimum figure. Very sensitive techniques, such as electrophoresis of protein subunits in sodium dodecyl sulfate-polyacrylamide gel, demonstrate 35 to 40 components, some of which make up as little as 0.15% of the total.<sup>90</sup> As yet, little is known about the relation of these protein fractions to the structure or function of the membrane. However, two proteins have been more fully characterized, and appear to have special physiologic or pathologic significance.

The protein that becomes soluble in the presence of EDTA and other chelating agents has been designated "spectrin" because of its origin from red cell "ghosts."<sup>83</sup> Accounting for about 20% of stromal protein, spectrin contains no carbohydrate or lipid groups and appears homogeneous by electrophoresis, ultracentrifugation, and immunodiffusion. Amino acid analysis shows it to be particu-

larly rich in glutamic acid and to contain cysteine. The monomer appears to have a molecular weight of about 140,000, but polymers of varying size will form, depending on pH, concentration, and the nature of the buffer. Of particular interest is the tendency of spectrin to form insoluble filaments or fibers in the presence of divalent cations, especially calcium. Studies with electron microscopy of membranes before and after spectrin extraction suggest that the protein is located on the inner surface of the erythrocyte membrane.

The physiologic importance of spectrin is suggested by observations indicating deficiency or malfunction of a spectrin-like protein in hereditary spherocytosis (HS)<sup>70</sup> (Chapter 21). Extracts of membrane protein from patients with HS fail to precipitate or form fibers to the same degree as do normal extracts when exposed to divalent cations or vinblastine. Since the membrane in HS is excessively permeable, spectrin may be essential to the permeability barrier that characterizes the normal membrane.

Another well-studied membrane protein is the principal glycoprotein (or closely related family of glycoproteins) of erythrocyte stroma.<sup>84,119</sup> This protein, for which the name "erythrocyte glycoporphin" has been suggested,<sup>81</sup> accounts for about 10% of membrane protein. Glycophorin is found in one of the detergent-soluble fractions of membrane protein,<sup>98</sup> and may also be isolated by other methods, including extraction with lithium di-iodosalicylate<sup>84</sup> or with hot, aqueous phenol solutions, or by chromatography on Sephadex G-200 with 33% pyridine as eluant. In detergent solutions a molecular weight of about 55,000 is observed, but aggregates with molecular weights as great as 500,000 may occur. About 60% of the molecule is carbohydrate, accounting for most of the sialic acid of the membrane and about half of the hexose and hexoseamine as well as small amounts of mannose and fucose.

The membrane glycoprotein is found on the external surface of the cell. It is likely that the lipophilic, C-terminal end is attached to membrane lipids, and the more soluble,



carbohydrate-containing N-terminal end projects into the erythrocyte environment. The glycoprotein accounts for the characteristic negative charge of the red cell, which may act to prevent excessive erythrocyte agglutination. Another feature of this protein is that it carries the A, B, M, and N specific blood group antigens as well as "receptors" for influenza viruses, phytohemagglutinin, and wheat-germ agglutinin.

Other membrane proteins include certain enzymes that seem to be firmly attached to the erythrocyte membrane. These include glyceraldehyde-3-phosphate dehydrogenase,<sup>64,108</sup> aldolase,<sup>64</sup> 3-phosphoglycerate kinase,<sup>61</sup> adenylate kinase,<sup>61</sup> cyclic AMP-dependent protein kinase,<sup>109</sup> ATPase (page 101), cholinesterase,<sup>216</sup> and a thyroid hormone deiodinating enzyme.<sup>93</sup>

### Membrane Transport Functions

In general, the membrane acts as a partial barrier to penetration of all solutes. In the case of nonpolar substances, the rate of diffusion through the membrane usually is proportional to their solubility in organic solvents. Polar solutes apparently cross the membrane at specialized sites or pores.

One exception to the above generalization regarding nonpolar solutes exists in relation to the monosaccharides.<sup>85</sup> These easily cross the membrane barrier, whereas the more lipid-soluble disaccharides do not. Of the common hexoses, glucose is transported most rapidly, followed by mannose, galactose, xylose, arabinose, and fructose. Highly specific binding sites for D-glucose can be demonstrated on the red cell membrane.<sup>77</sup> In contrast to certain other tissues, the erythrocyte does not require insulin for penetration of glucose, nor is there any effect of thyroid or pituitary hormones. Glucose transport does not occur against a gradient, nor does it require energy. On the other hand, simple diffusion cannot explain the speed nor the kinetic pattern of uptake.<sup>85</sup> The conventional explanation for this type of facilitated diffusion is that monosaccharides combine with a membrane constituent, usually termed a "carrier,"

which then becomes reoriented toward the opposite side of the membrane and discharges its glucose into the cell. However, certain experimental observations are not easily explained by the carrier model, and non-carrier models currently are under study.<sup>80</sup>

Of the important polar substances, water and most anions, especially chloride and bicarbonate, diffuse freely and passively across the membrane. In contrast, the major monovalent cations, sodium and potassium, require an energy-dependent transport mechanism. This important process maintains the internal osmotic environment and preserves the normal gradients between plasma and intracellular concentrations of sodium and potassium.

Within the human erythrocyte, potassium is the predominant cation and sodium is a relatively minor constituent, whereas the relationship is reversed in plasma (Fig. 3-13). Unlike nerve and muscle cells, erythrocytes have no known function that requires maintenance of this cation relationship, in fact, some species, eg, dogs and cats, have an intracellular cation environment more nearly like that of plasma. However, the mechanism that maintains the electrolyte relationship also regulates the intracellular volume. If active cation transport is paralyzed, the cells accumulate sodium and water until a critical volume is reached, usually about 1.5 times normal, and then hemolysis ensues with discharge of cellular constituents into the surrounding space.

The steady-state cation concentrations within the erythrocyte are the result of an equilibrium between passive diffusion ("leak") and active transport ("pump").<sup>110</sup> In sodium, the direction of "leak" is inward and the direction of "pump" is outward; in contrast, potassium leaks out and is pumped in.

From *in vitro* studies it was proposed that three sodium pumps exist in the red cell (Table 3-3).<sup>74</sup> Two of these, designated Ia and Ib, are similar in that they require adenosine triphosphate (ATP) and are inhibited by cardiac glycosides such as ouabain. They differ from one another in that Ia requires potassium in the external medium and Ib requires sodium. Pump II is not inhibited by ouabain,

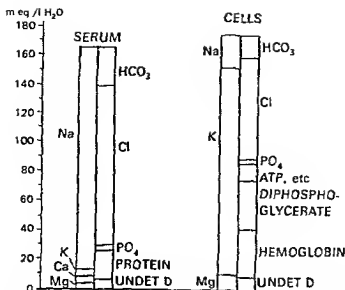


Fig 3-13 The intracellular electrolyte composition of the erythrocyte as compared with serum (From Guest<sup>70a</sup> courtesy of the author and American Journal of Diseases of Children)

but is inhibited by ethacrynic acid. The immediate energy source for pump II is unknown; it is not ATP, but glucose metabolism is required for its production. Others have presented evidence that "pump II" represents exchange diffusion, ie, a bidirectional flux that achieves no net sodium transport and, therefore, is not a "pump."<sup>63</sup> In any event, the most important pump is pump Ia, which exchanges sodium for potassium. The stoichiometry of the process is such that, for each molecule of ATP converted to ADP, three sodium ions are pumped out and two potassium ions enter.<sup>67</sup> One anion, usually chloride, leaves the cell passively to maintain elec-

trical neutrality. Ouabain-sensitive potassium exchange (K for K) has also been described in erythrocytes.<sup>100</sup>

Active cation transport appears to depend on a membrane-associated enzyme, adenosine triphosphatase (ATPase).<sup>62,78</sup> The enzyme may, in fact, be identical with the pump itself. It is this enzyme system that is inhibited by cardiac glycosides, and studies with these inhibitors suggest that there is a maximum of 250 sites on each red cell at which ATPase-dependent cation transport occurs. ATPase also is inhibited severely by low temperatures, accounting for the cellular potassium loss occurring during cold storage of blood.<sup>120</sup> The ATPase reaction may be divided into two essential steps: sodium- and magnesium-dependent phosphorylation, during which ATP is converted to ADP, and a potassium-dependent phosphatase reaction. Whether both are catalyzed by a single enzyme, or whether a structurally associated, double enzyme system is involved is not known. The reaction appears to require an intermediate, which is phosphorylated at the sodium-dependent step. The intermediate has not been fully characterized, and may be the enzyme itself; however, phosphatidylserine is required for optimal ATPase activity<sup>178</sup> and may be part of a cation carrier lipoprotein

Table 3-3. Sodium "Pumps" in the Erythrocyte

	Ia	Ib	II
Proportion of total Na flux (%)	50	20	25
Cation requirement in external medium	K	Na	Na
Energy source	ATP	ATP	?
Inhibition	ouabain	ouabain	ethacrynic acid

\*From Hoffman,<sup>74</sup> courtesy of the author and the American Journal of Medicine

that is powered by the reaction. The molecular weight of ATPase has been estimated at 300,000 to 400,000.<sup>62</sup>

### Glucose Metabolism

Although the mature red cell contains the enzymes required for glycogen metabolism, the balance between synthesis and utilization is such that no significant amount of glycogen accumulates within the cell.<sup>200</sup> Consequently, for its continued metabolism the erythrocyte must have constant access to glucose. As previously discussed (page 100), glucose enters the cell by a process of "facilitated transport," perhaps utilizing a carrier mechanism. There is no requirement for insulin or other hormones.

Lacking mitochondria, erythrocytes must depend on two much less efficient pathways for production of high-energy compounds. One of these is anaerobic glycolysis (Embden-Myerhof pathway), whereby glucose is broken down through a series of phosphorylated intermediates to lactate (Fig. 3-14, reactions 1 to 11). About 90% of glucose entering the red cell follows this route.<sup>201</sup> The enzymes catalyzing these reactions have all been found in erythrocytes, and normal values have been reported by several groups.<sup>171-217</sup>

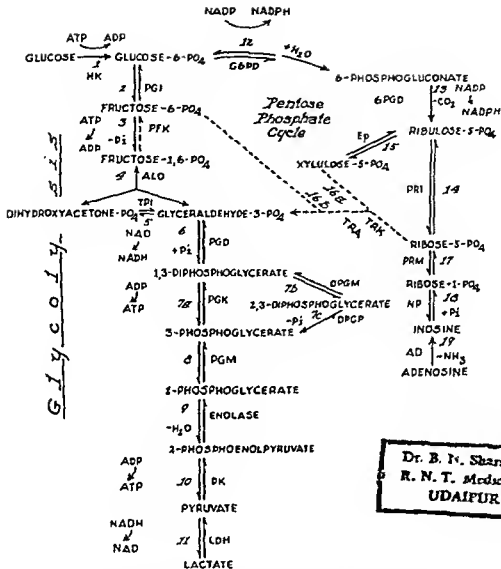
Two important products are formed during anaerobic glycolysis. One of these is reduced nicotinamide-adenine dinucleotide (NADH, DPNH) (Fig. 3-14, reaction 6) a compound essential for the reduction of methemoglobin (page 105). The other is the major high-energy compound in erythrocytes, adenosine triphosphate (ATP). For each mole of glucose catabolized, two moles of ATP are utilized (Fig. 3-14, reactions 1 and 3) and a maximum of four may be produced (two moles in reaction 7a and two in reaction 10). Thus, at maximum efficiency, a net yield of two moles of ATP may be expected for each mole of glucose completing the series of reactions. This net yield may be decreased, however, by a side pathway unique to the red cell, the 2,3 diphosphoglycerate (2,3 DPG) pathway (Fig. 3-14, reactions 7b and 7c), also known

as the Rapoport-Luebering shunt after its discoverers.<sup>209</sup>

The principal phosphorylated intermediate in erythrocytes is 2,3 DPG, accounting for about two thirds of the red cell phosphorus. In contrast, 2,3 DPG is present in only trace amounts in other tissues. The production of 2,3 DPG constitutes a sort of "energy clutch" because it diverts 1,3 DPG from a step in which ATP is synthesized (reaction 7a, Fig. 3-14) to one in which a high-energy phosphate is "wasted" (reaction 7b). The proportion of 1,3 DPG following the ATP pathway as opposed to the 2,3 DPG pathway appears to be related largely to cellular ADP and ATP levels; when ATP falls and ADP rises, a greater proportion of 1,3 DPG is converted through the ATP-producing step. This mechanism serves to assure a supply of ATP to meet cellular needs.

Early theories for the function of 2,3 DPG in the red cell stressed its role as a buffer and as a short-term storage compound that could be utilized during periods of glucose deprivation. It is now apparent that 2,3 DPG has another role unrelated to energy metabolism; it affects tissue oxygen delivery by reversibly combining with deoxygenated hemoglobin and decreasing hemoglobin oxygen affinity (page 107).

The other pathway of erythrocyte glucose metabolism is the aerobic, pentose phosphate pathway (PPP; also known as the hexose monophosphate shunt or the phosphogluconic acid oxidative pathway) (Fig. 3-14, reactions 12 to 15). Under normal circumstances about 10% of red cell glucose is metabolized aerobically.<sup>201</sup> For many years it has been known that the proportion of glucose metabolized in the erythrocyte via the PPP could be greatly increased *in vitro* by adding methylene blue. Methylene blue has no clear physiologic equivalent; however, a number of other substances may have a similar effect, including cysteine, ascorbate, pyruvate, primaquine, and others.<sup>174,226</sup> The relative activity of the PPP appears to be regulated largely by the concentration of oxidized glutathione (GSSG); when GSSG concentration increases, there is a corresponding increase in



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Fig. 3-14. Pathways for the metabolic breakdown of glucose in the red corpuscle and some of the enzymes involved. The interrupted lines indicate that some steps have been omitted. HK, Hexokinase; PG1, phosphoglucose isomerase; PFK, phosphofructokinase; ALO, aldolase; TPI, triosephosphate isomerase; PGD, phosphoglyceraldehyde dehydrogenase (glyceraldehyde phosphate dehydrogenase); PGK, phosphoglyceric acid kinase; PGM, phosphoglyceromutase; DPGM, diphosphoglyceratemutase; DPGP, diphosphoglycerate phosphatase; PK, pyruvate kinase; LDH, lactic dehydrogenase; G6PD, glucose-6-phosphate dehydrogenase; 6PGD, 6-phosphogluconic dehydrogenase; PRI, phosphonbomutase; Ep, epimerase; TRK, transketolase; TRA, transaldolase; AD, adenosine deaminase; NP, nucleoside phosphorylase; PRM, phosphonbomutase. (Prepared with the advice of Dr. G. W. E. Plaut.)

the amount of glucose that enters the PPP.<sup>186</sup>

The principal high-energy product of aerobic glucose metabolism via the PPP is reduced nicotinamide-adenine dinucleotide phosphate (NADPH, TPNH) (Fig. 3-14, reactions 12 and 13). The erythrocyte lacks the machinery to utilize NADPH for energy; instead, NADPH serves as a cofactor in the reduction of glutathione.

### Glutathione Metabolism

In order to survive, the red cell must be able to protect hemoglobin, enzymes, and the membrane from oxidative damage. This function is performed by a system in which reduced glutathione is a key component. Other components of the system include NADPH and the enzymes glucose-6-

ted activity.<sup>189</sup> The investigators suggested that the NADH and NADPH methemoglobin reductases represented different sites on single proteins rather than distinct enzymes. This conclusion is difficult to accept, however, since NADH- and NADPH-methemoglobin reductases can be separated electrophoretically.<sup>184</sup> Also, distinct hereditary syndromes affecting one system but not the other would be difficult to explain on this basis.

### Miscellaneous Enzymes in Red Corpuscles

In addition to the enzymes involved in the glycolytic cycle and in the pentose phosphate pathway, listed in Figure 3-14, many others have been identified and quantitated.<sup>154,162,183,178,193,219,224,229,233</sup> These include: (1) Remnants of Krebs cycle enzymes, including fumarase,<sup>215</sup> malic dehydrogenase,<sup>221</sup> aconitase,<sup>161</sup> and isocitric dehydrogenase.<sup>215</sup> (2) Carbonic anhydrase,<sup>155,211,227</sup> a zinc enzyme occurring in two forms (CA I and CA II), each under independent control of distinct autosomal genes.<sup>227</sup> Carbonic anhydrase catalyzes the reversible formation and dissociation of carbonic acid, thereby contributing to CO<sub>2</sub> transport (page 108). (3) "True" cholinesterase, a membrane-associated enzyme that differs from the relatively nonspecific cholinesterase found in plasma. Cholinesterase is found in far higher concentration in reticulocytes and young red corpuscles than in older ones.<sup>216</sup> (4) Proteolytic enzymes<sup>199</sup> and peptidases.<sup>151</sup> (5) Purine nucleoside phosphorylases specific for nucleosides such as inosine and guanosine.<sup>229,232</sup> (6) Nicotinamide ribonucleoside phosphorylase.<sup>180</sup> (7) Arginase,<sup>157,210</sup> glyoxylase,<sup>175</sup> urease and a thiocyanate oxidase.<sup>179</sup> (8) Adenylate kinase.<sup>167</sup>

Since the production of enzymes is a genetically determined phenomenon, it is to be expected that mutations affecting the activity of various enzymes should occur. Some of these, such as acatalasemia<sup>152,157</sup> and 6-phosphogluconate dehydrogenase deficiency,<sup>165</sup> are associated with no demonstrable abnor-

malities of the red corpuscle. Others may be lethal, thus preventing their recognition. One might have postulated that this would be the case with hexokinase, an enzyme that plays a key role in the metabolism of glucose (Fig. 3-14, reaction 1). This, however, does not appear to be true regarding hexokinase deficiency, and a number of other genetically determined enzyme deficiencies associated with hematologic abnormalities but not necessarily lethal have been discovered. The abnormalities include congenital hemolytic anemias (pyruvate kinase, glucose-6-phosphate dehydrogenase, glutathione reductase, diphosphoglycerate mutase, and triosephosphate isomerase) and hemolytic anemias associated with drug ingestion (G6PD, glutathione reductase). These will be discussed in Chapters 22 and 23.

### Function of the Erythrocyte

One of the most striking examples of the importance of the evolutionary process, as well as of the efficiency of the body economy, is found in the mammalian erythrocyte. Essential to mammalian life is a process of combustion for which a constant supply of oxygen and the simultaneous removal of carbon dioxide are required. The function of the red cell is to mediate the exchange of respiratory gases, oxygen and carbon dioxide, between the lungs and the tissues. In man at rest, about 250 ml of oxygen are absorbed and 200 ml of carbon dioxide are produced per minute. During exercise these quantities increase tenfold. If the respiratory gases were carried in physical solution in the plasma, man's activity could only be one-fiftieth of that actually possible. The development of hemoglobin makes possible the transportation of a hundred times as much oxygen as could be carried by the plasma alone.

In the majority of invertebrates, oxygen-carrying pigment is transported freely in the plasma rather than within cells.<sup>196</sup> This is a relatively inefficient method. Hemoglobin, as a protein free in the plasma, exerts an osmotic pressure about five times greater than that produced by the plasma proteins. By

the inclusion of this pigment in corpuscles the viscosity of the blood is maintained at a low level, water is not drawn from the tissues by it, and the flow of blood containing such a large amount of protein is made possible.

When fully saturated, each gram of hemoglobin binds 1.34 ml of oxygen. The degree of saturation is related to the oxygen tension of the blood, which normally ranges from 100 mm Hg in arterial blood to about 35 mm Hg in veins. The relation between oxygen tension and hemoglobin oxygen saturation is described by the oxygen-dissociation curve of hemoglobin (Fig. 3-16). The characteristics of this curve are related in part to properties of hemoglobin itself and in part to the environment within the erythrocyte, including pH, temperature, ionic strength, and concentration of phosphorylated compounds, especially 2,3 diphosphoglycerate (2,3 DPG).

Oxygen affinity of hemoglobin is generally expressed in terms of the oxygen tension at which 50% saturation occurs, the so-called  $P_{50}$ . When measured in whole erythrocytes, this value averages 27.1 mm Hg in normal, non-smoking males and 27.5 mm Hg in normal, non-smoking females.<sup>228</sup> When oxygen affinity is increased, the dissociation curve is shifted leftward, and the value for  $P_{50}$  is

reduced. Conversely, with decreased oxygen affinity, the curve is shifted to the right and  $P_{50}$  is increased.

With heme polypeptides consisting of a single subunit (eg, myoglobin) the oxygen-dissociation curve is hyperbolic, and oxygen affinity is considerably greater than that of hemoglobin (Fig. 3-16). Such a compound would function poorly in oxygen transport because little oxygen would be released until the tissue oxygen tension became very low. In contrast, the oxygen-dissociation curve of hemoglobin is distinctly sigmoidal, and the steepest part of its slope occurs at levels of oxygen tension corresponding to those found in tissues. This difference between the hemoglobin and myoglobin curves is the result of interaction between the four heme-polypeptide units of hemoglobin (page 176). This phenomenon commonly is referred to as "heme-heme interaction"; however, since no fundamental chemical change occurs in the heme group itself, perhaps the term "subunit interaction" is to be preferred.<sup>229</sup> In essence, subunit interaction leads to a change in affinity for oxygen as each of the four  $O_2$ -binding sites becomes occupied. Affinity for the first  $O_2$  molecule is relatively low, and affinity increases with each molecule bound.

The change in oxygen affinity with pH is known as the Bohr effect.<sup>212</sup> Hemoglobin oxygen affinity is reduced as the acidity increases (Fig. 3-16). Since the tissues are relatively rich in carbon dioxide, the pH is lower than in arterial blood; therefore, the Bohr effect facilitates transfer of oxygen. The Bohr effect is a manifestation of the acid-base equilibria of hemoglobin. The dissociation constant ( $pK$ ) of the reaction  $HHb \rightleftharpoons H^+ + Hb$  is 7.9, whereas after oxygenation, the  $pK$  decreases to 6.7. These changes are important not only for oxygen delivery, but also in carbon dioxide transport (see below).

Another important factor affecting the oxygen affinity of hemoglobin is the concentration of phosphorylated compounds, especially 2,3 DPG.<sup>158,166,168,170</sup> (see page 102). In the deoxygenated state, hemoglobin can bind 2,3 DPG in a molar ratio of 1:1, a reaction leading to reduced oxygen affinity.

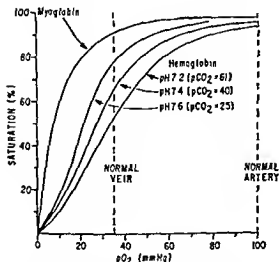


Fig. 3-16. Oxygen-dissociation curve of hemoglobin, at three values for pH, compared with that of myoglobin.  $pO_2$ , Partial pressure of oxygen,  $pCO_2$ , partial pressure of carbon dioxide

The increased oxygen affinity of fetal hemoglobin appears to be related to its lessened ability to bind 2,3 DPG.<sup>231</sup>

The increased oxygen affinity of stored blood is accounted for by reduced levels of 2,3 DPG.<sup>169</sup> Transfusion of such blood results in an in vivo increase in oxygen affinity that returns toward normal in several hours as the function of the glycolytic pathway is restored. The reduction in 2,3 DPG levels in stored blood can be mitigated by adding inosine or phosphate to the storage solutions.<sup>206</sup>

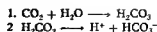
Changes in 2,3 DPG levels play an important role in adaptation to hypoxia. In a number of situations characterized by hypoxemia, red cell 2,3 DPG levels increase, oxygen affinity is reduced, and delivery of oxygen to tissues is facilitated. These include abrupt exposure to high altitude,<sup>195</sup> anoxia due to pulmonary or cardiac disease,<sup>203,235</sup> blood loss,<sup>176</sup> and anemia.<sup>225</sup> Increased 2,3 DPG also plays a role in adaptation to exercise.<sup>205</sup>

The factors controlling erythrocyte 2,3 DPG levels have not been defined completely. However, probably the most important one is the overall rate of glycolysis.<sup>213,228</sup> Glycolysis is stimulated by alkalosis and depressed by acidosis,<sup>158,171</sup> probably because of an effect of pH on phosphofructokinase (Fig. 3-14, reaction 3).<sup>207</sup> Thus, the respiratory alkalosis that occurs with ascent to high altitude could lead to increased rates of glycolysis and increased 2,3 DPG levels. Also, intracellular alkalosis might occur with excessive hemoglobin deoxygenation because of the Bohr effect. Another possible mechanism is diversion of 1,3 DPG to the 2,3 DPG pathway (Fig. 3-14, reaction 7b). Such a diversion might be accomplished by an increase in deoxygenated hemoglobin, which in turn binds 2,3 DPG and relieves end product inhibition of 2,3 DPG mutase.<sup>204</sup>

Molecular mechanisms for explaining subunit interaction, the Bohr effect, and the 2,3 DPG effect are discussed in Chapter 4 (page 176).

Transport of carbon dioxide by red cells, unlike that of oxygen, does not occur by direct binding to heme.<sup>225</sup> In aqueous solutions, carbon dioxide undergoes a pair of

reactions:



Carbon dioxide diffuses freely into the red cell where the presence of the enzyme *carbonic anhydrase* facilitates reaction 1. The  $\text{H}^+$  liberated in reaction 2 is accepted by deoxygenated hemoglobin, a process facilitated by the Bohr effect. The bicarbonate formed in this sequence of reactions diffuses freely across the red cell membrane and a portion is exchanged with plasma  $\text{Cl}^-$ , a phenomenon called the "chloride shift." About 70% of tissue carbon dioxide is processed in this way. Of the remaining 30%, 5% is carried in simple solution and 25% is bound to deoxygenated hemoglobin amino acid groups, forming carbamino-hemoglobin.

Alterations in the oxygen dissociation curve occurring in various hemoglobinopathies will be discussed in Chapter 30 (page 982).

### Erythrocytes of Various Mammals and Lower Vertebrates

Differences in number, size, and morphologic characteristics of the red blood corpuscles of the lower vertebrates and those of the mammalia furnish further evidence of the efficiency attained through the evolutionary process. With the exception of the antarctic ice fish and the larvae of certain eels, all vertebrate species possess erythrocytes.<sup>197</sup> Most vertebrate red cells are nucleated and have a capacity for aerobic, oxidative metabolism. The occurrence of a denucleated cell that is dependent on anaerobic glycolysis for energy and is rich in 2,3 DPG is a late evolutionary development, characteristic of mammals.

Differences in the mass of red corpuscles, as represented by the volume of packed red cells, are relatively small throughout the vertebrate phylum<sup>234</sup> (Fig. 3-17). Correspondingly, the range in amount of hemoglobin per unit volume of blood is small, being actually no greater than that found in anemia. There is, in other words, a relatively constant

Mean Corpuscular Hemoglobin Concentration

Mean Corpuscular Hemoglobin

Mean Corpuscular Volume

Volume of Packed Red Cells

Hemoglobin

Number of Red Corpuscles

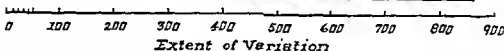


Fig. 3-17. The red corpuscles of various vertebrates vary greatly in number per liter as well as in mean corpuscular volume and mean corpuscular hemoglobin. However, there is comparatively little variation in volume of packed red cells or in blood hemoglobin concentration and almost none in the mean corpuscular hemoglobin concentration.

amount of hemoglobin available for the transportation of oxygen in most vertebrates. However, the differences in the number and kind of vehicles that transport this hemoglobin are extraordinarily great. In the vertebrate phylum, erythrocyte counts as low as  $0.021 \times 10^{12}/l$  (in a tailed amphibian, *Amphiuma*) and as high as  $17.9 \times 10^{12}$  (in the goat) have been found.<sup>234</sup> The size of the red corpuscles is inversely proportional to the red cell count, the corpuscles of one of the tailed amphibia measuring almost 15,000 fl, while those of the goat measure 19 fl. In Figure 3-18, the red corpuscles of human blood and those of the "Congo Snake," a tailed amphibian, are compared. (See also Table B-2, Appendix B.)

The excessively large corpuscles of the lower vertebrates probably are transported less readily through the blood vessels than are the smaller corpuscles of mammalia. What is still more important, the small size of the corpuscles of mammals is adapted for the maximal development of surface. For the gaseous exchanges that occur through the

mediation of the erythrocyte, this is extremely important.<sup>183</sup>

The biconcave shape of the erythrocyte has been shown to be the best shape whereby in- and out-going gases may reach and leave all parts of the corpuscle with the least variation in time.<sup>181</sup> The efficiency of the mammalian red cell is favored by its plasticity<sup>101</sup> and the absence of a nucleus. Although the red cell is by no means metabolically inert, as was once assumed (see page 102) its oxygen consumption is only 0.5% of that of nucleated red cells<sup>190</sup> and less even than that of reticulocytes.<sup>208</sup> Thus it consumes but little of the gas it transports.

## Laboratory Evaluation of Erythrocytes

### Measures of Concentration

Determination of the concentration of red cell elements in the blood is required for the purpose of detecting anemia or polycythemia



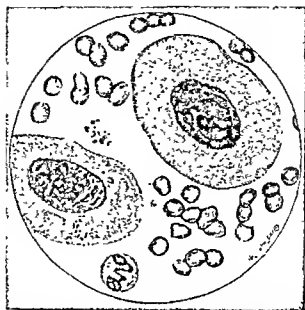


Fig 3-18 The giant nucleated erythrocytes of a tailed amphibian (*Amphiuma means*) compared with human red corpuscles. A polymorphonuclear leukocyte is also shown (Drawing of a slide stained with Wright's and magnified  $\times 960$ )

or in order to calculate erythrocyte indices (page 115). The measures of concentration fall into three groups: those for determining the volume of packed red cells (VPRC), the blood hemoglobin concentration (Hb), and the red cell count (RBC). Red cell counting methods are discussed in Chapter 1 (page 9).

### Volume of Packed Red Cells

The VPRC is determined by centrifugation of blood in a container known as a hematocrit. Various early types of hematocrits were introduced in the latter part of the 19th century by Hedin,<sup>329</sup> Blik, and Daland.<sup>316</sup> These instruments, as well as that designed by Van Allen,<sup>326</sup> were inaccurate, partly because leakage often occurred at the distal ends during centrifugation and also because optimal conditions for time and force of centrifugation were not established. These sources of error were eliminated in 1929 by Wintrobe hematocrit (Fig. 3-19) and the specifications established for its use. Methods

currently in use for determining the VPRC include the original Wintrobe or macro method and more recently described micro methods. In addition, approximations of the "hematocrit" can be made by determining the conductivity of blood or by calculations based on the number and size of red cells as determined with an electronic particle counter. The last two methods are described in Chapter 1 (pages 14 and 20).

In the Wintrobe (macro) method the hematocrit is filled with anticoagulated blood by means of a capillary pipet and centrifuged for 30 minutes. The object of centrifugation is to secure optimal packing of the erythrocytes so that a minimal and reproducible amount of plasma remains between them, and yet distortion or expulsion of some of their contents does not occur. For this purpose, a relative centrifugal force (RCF) of  $2260 \times$  gravity (G) is employed. RCF depends upon the distance of the particle from the center of revolution (radius,  $r$ ) as well as upon the speed of centrifugation in revolutions per minute (RPM). This relationship is

indicated by the formula:

$$RCF = 0.000,011,18 \times r \times (RPM)^2$$

The radius ( $r$ ) is defined as the distance (in cm) from the center of the drive shaft of the centrifuge to the bottom of the hematocrit tube as it is held horizontally in the centrifuge cup. The method was standardized with a No. 2 International Centrifuge and a centrifuge head with a radius of 22.5 cm, which, at 3000 RPM will produce an RCF of  $2264 \times G$ . Since centrifuge heads with quite different radial distances may be used, it is necessary to determine, for each, the speed that must be attained to produce the desired RCF. Substituting in the formula given above an RCF of  $2260 \times G$ , the equation may be

written:

$$RPM = \sqrt{\frac{202,146,700}{r \text{ (cm)}}}$$

Thus, by measuring the radius of a particular centrifuge head, one may calculate the speed of centrifugation required to achieve the necessary RCF. The RPM should be determined by means of a tachometer since the attainable speed indicated on the face of the centrifuge is not necessarily correct.

Maximal packing of the erythrocytes is achieved after 30 minutes of centrifugation. Prolongation of the time of centrifugation does not compensate for an inadequate RCF. Unless the centrifuge is capable of producing the number of revolutions per minute required by its radius to attain the necessary RCF, centrifugation even for several hours will not produce optimal packing.

The VPRC may be read directly from the scale embossed on the Wintrobe tube, which is divided into centimeters and millimeters. If the hematocrit has been filled to "10" (Fig. 3-20), the level at which the packed red corpuscles are found, if multiplied by 0.1, will give the volume in liters per liter (l/l) of blood.

At the uppermost level of the packed red cells, immediately adjacent to the reddish-gray layer of packed white corpuscles, a narrow black band will be seen. This represents a layer of erythrocytes in which the oxyhemoglobin has been reduced by the metabolic activity of the leukocytes.<sup>301</sup> The reading of "volume of packed red cells" is made at the uppermost level of the black line.

Determination of the VPRC in the Wintrobe tube has the advantage of yielding useful additional information with little extra effort. This includes the sedimentation rate (ESR, page 125), the icterus index (Chapter 5, page 214), and the volume of packed white cells and platelets. Above the deep red layer of packed red corpuscles, a reddish-gray layer of packed leukocytes and platelets is found (Fig. 3-20). In normal blood this ranges from 0.5 to 1 mm in thickness, each 0.1 mm corresponding approximately to  $1.0 \times 10^9$ /leuko-



Fig. 3-19. Wintrobe's hematocrit, pipet and bulb used for filling it, and cap for hematocrit. Two thirds actual size



**Fig 3-20** Photographs of hematocrits containing blood from patients with leukemia and polycythemia. **A**, Chronic lymphocytic leukemia: volume of packed WBC and platelets 0.265 l per liter of blood. VPRC, 0.205 l. The leukocyte count was  $900 \times 10^9/l$ , platelets numbered  $180 \times 10^9/l$  erythrocytes  $2.33 \times 10^{12}/l$ . **B, C**, Chronic myelocytic leukemia. The respective erythrocyte counts were 4 and  $2.3 \times 10^{12}/l$  but the leukocyte and platelet counts were approximately the same (WBC  $42 \times 10^9/l$  and  $35 \times 10^9/l$  respectively, platelets  $360 \times 10^9/l$  and  $325 \times 10^9/l$ ). **D**, Erythremia showing an increase in all the corpuscles of the blood. RBC  $5.7 \times 10^{12}/l$ , WBC  $24.4 \times 10^9/l$ , platelets  $430 \times 10^9/l$ . The successive layers of packed platelets, leukocytes, and red corpuscles are particularly clear in **B, C**, and **D**.

cytes. Thus, when the platelet count is approximately normal, the thickness of this reddish-gray layer may be used as a rough index of the leukocyte count. When the leu-

kocyte count is greater than  $12.0 \times 10^9/l$  the correlation between the thickness of the leukocyte layer and the white cell count is less accurate than when the count is below this value; but even for counts as high as  $30.0 \times 10^9/l$  the thickness of the reddish-gray layer is a remarkably useful guide. When leukocytosis is marked, relatively more packing of leukocytes tends to occur than in normal blood and 0.1 mm corresponds more nearly to  $2.0 \times 10^9/l$  than to  $1.0 \times 10^9/l$ .

The thickness of the layer above the packed red corpuscles depends on the number of leukocytes, the kind of leukocytes, and the quantity of platelets. Since lymphocytes are smaller than the cells of the myeloid series, when there is relative lymphocytosis the layer will be narrower for a given quantity of corpuscles than when myeloid leukocytes predominate. Again, if the platelets are reduced in number the layer is correspondingly narrower. In leukopenia, and particularly when this is accompanied by thrombocytopenia, the layer of corpuscles above the red cells is barely perceptible. On the other hand, when the platelets are more numerous than usual, and sometimes even when they are present in normal numbers, it is possible to distinguish two portions in the layer above the red corpuscles (Fig. 3-20, B, C, D). Uppermost will be found a cream-colored layer which, on aspiration smear and microscopic examination (Fig. 3-21), is found to consist practically entirely of platelets. The reddish-gray layer below this consists almost exclusively of leukocytes (Fig. 3-22). In chronic myelocytic leukemia and in erythremia, in which both the number of leukocytes and the quantity of platelets are increased, the three well-defined layers of corpuscles present a striking picture (Frontispiece, Plate I). Separation of the corpuscles into layers is aided by a period of sedimentation preceding centrifugation.

Observation of the color and opacity of the blood plasma will reveal degrees of jaundice that are not sufficiently marked to be perceived by physical examination. The *icterus index* of the plasma is easily measured in the hematocrit tube by comparison with stand-

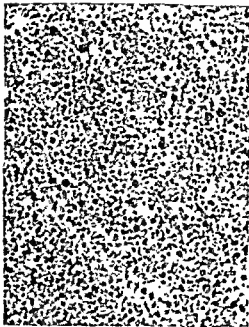


Fig. 3-21. Microscopic view of the topmost cream-colored layer in the hematocrit shown in Figure 3-20. C It is composed almost entirely of platelets ( $\times 700$ )

ards in tubes of the same size (Chapter 5, page 215). Lipemia also is easily detected, the plasma appearing quite opaque in this condition. Other plasma abnormalities may be discovered occasionally, such as cryoglobulins in multiple myeloma.<sup>352</sup>

The micro method for determining

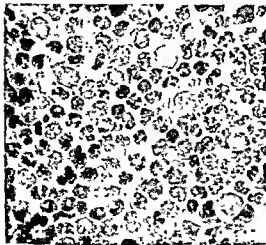


Fig. 3-22. The reddish-gray layer below the cream-colored layer in the hematocrit shown in Figure 3-20. B and D. It is composed almost entirely of leukocytes ( $\times 700$ )

VPRC<sup>310</sup> has the advantage of requiring smaller amounts of blood, shorter centrifugation times, and smaller, less expensive centrifuges than the macro method. The determination is performed in capillary tubes that are of uniform bore and usually open at both ends.<sup>337,339</sup> The tubes usually are sealed with clay (less often, with heat) prior to centrifugation. Tubes containing dried heparin are available so that capillary blood may be used without prior anticoagulation. A variety of microhematocrit centrifuges can be obtained from commercial sources; most of them are small, portable, and inexpensive. These instruments exert a RCF of 11,000 to 15,000  $\times G$ , but speed is not controllable and no tachometer is provided. The only control is a time switch. Maximal packing usually is achieved after five minutes of centrifugation. The microhematocrit tubes have no scale of their own and must be inspected with any of several calibrated devices in order to read the VPRC. Because of the high RCF, the value usually is slightly lower (0.01 to 0.03 l/l) than that obtained with the macro method.

The micro methods have certain disadvantages as compared with the standard Wintrobe method. Most important of these is the reading error which, although negligible at normal levels for the VPRC, approaches 5% ( $\pm 2$  CV) at low levels.<sup>310</sup> Additional variability may be introduced by the use of capillary blood samples. Furthermore, the separation of leukocytes from the packed red cell column is less complete, especially when there is marked leukocytosis. The microhematocrit is therefore less reliable than the macro method for calculation of the erythrocyte indices.<sup>310</sup> Finally, sedimentation rate, icterus index, and the volume of packed white cells and platelets cannot be determined in the capillary tubes. These disadvantages tend to be minor ones, however, and for the routine detection of anemia and polycythemia as well as for the serial observation of fluctuations in these findings, the method is satisfactory<sup>224</sup> and is particularly well suited to the small laboratory, such as that in many doctor's offices.

### Accuracy of Hematocrit Methods

For the VPRC to be a perfectly accurate indication of the proportional volume of blood occupied by erythrocytes, the conditions of centrifugation would have to be such that no plasma remains in the red cell column, and yet none of the cellular contents is expressed.

Plasma trapping has been measured by dilution of a plasma label, such as  $^{131}\text{I}$ -albumin. These studies have demonstrated that when centrifugation is carried out as specified for the Wintrobe (macro) method, an average of 2.13% of the red cell column represents trapped plasma.<sup>319</sup> This amount may vary somewhat at different VPRC values; thus, no plasma trapping could be demonstrated at values less than 0.33 l/l, but there was 2% trapping at 0.50 l/l and 4% at 0.68 l/l.<sup>319</sup> These differences presumably reflect the fact that the effective radius, and therefore the RCF, vary somewhat with the height of the red cell column. Somewhat more plasma is trapped if the red cells are spherocytes.<sup>323</sup>

For the purposes of detecting anemia or calculating erythrocyte indices a correction for trapped plasma usually is not made. For these purposes the important considerations are that the precision of the method is high and that the normal values in use today were established without such corrections. How-

ever, allowances for trapped plasma often are made when the VPRC is used in calculating total blood volume (page 122).

As previously noted, the values for VPRC obtained with the micro method are slightly lower than with the macro method because of the greater centrifugal forces applied in the micro method. Surprisingly, however, the amount of plasma trapping in microhematocrits was found to be of the same order of magnitude as that found with the Wintrobe method.<sup>321</sup> An increased amount of plasma trapping was found in some anemias, especially hypochromic anemias and sickle-cell anemia.

### Hemoglobin Concentration

Prior to 1959, blood hemoglobin concentration was measured by a variety of methods.<sup>310,320,331,332,333,339,344,348,353</sup> These have been superseded in almost all laboratories by spectrophotometric or colorimetric assays based on the conversion of hemoglobin to cyanmethemoglobin.<sup>306,310,315</sup> The major reason for the nearly universal acceptance of the cyanmethemoglobin method is the availability of stable, accurate standards.<sup>309</sup> Standards that carry the certification of the College of American Pathologists are available from several commercial sources. These have an approximate content of 60 mg cyanmethemoglobin per deciliter (dl) of solution,

Table 3-5. Comparison of Methods for Measuring Concentration of Red Cell Elements in Blood<sup>310</sup>

Method	Error <sup>1</sup>	Blood Required	Time Required (Min)	Equipment Cost	Additional Data Available
VPRC (macro)	1.9%	1.0 ml	35	High	Yes <sup>3</sup>
VPRC (micro)	2% <sup>2</sup>	Drop	10	Moderate	No
Hb (cyanmethemoglobin)	1.2%	Drop	20	High	No
Hb (Sahl's or Spencer)	10%	Drop	10-30	Low	No
RBC (hemocytometer)	9%	Drop	30	Low	No
RBC (electronic method)	3.6%	Drop	15	Highest	Yes <sup>4</sup>

Abbreviations: VPRC, volume of packed red cells; Hb, blood hemoglobin concentration; RBC, red cell count

1 Plus or minus 2 coefficients of variation, as performed by experienced hematologic technicians

2 At normal levels—increases to about 5% at low values

3 Sedimentation rate, icterus index, volume of packed leukocytes and platelets

4 Depending on the instrument size, distribution curves, automatic MCV calculation, and VPRC calculation from Cartwright<sup>310</sup> courtesy of the author and Grune & Stratton

corresponding to the dilution used in most clinical laboratories (1:251 of blood with a hemoglobin content of 15.0 g/dl). The standards can be used for at least a year from the date of production. Another advantage of cyanmethemoglobin is its broad absorption band in the region of 540 nm. The millimolar extinction coefficient of cyanmethemoglobin at this wavelength is taken to be 44.0.<sup>343</sup> A wide variety of satisfactory spectrophotometers and filter photometers are available.

To prepare cyanmethemoglobin, hemoglobin is reacted with ferricyanide and cyanide. Ferricyanide converts hemoglobin to methemoglobin, which then combines with cyanide to form cyanmethemoglobin. These two reactions are rapid and stoichiometric. The usual diluent in routine laboratories is Drabkin's solution, which consists of 1.0 g  $\text{NaHCO}_3$ , 50 mg potassium cyanide, and 200 mg potassium ferricyanide in 1.0 l distilled water. This is a clear solution, pale yellow in color. It should be kept in a brown bottle and a fresh solution should be made up once a month. The concentration of cyanide in the reagent is so low that as much as 4 l would be required to produce a lethal effect in man.

An accurately measured volume of blood is diluted in an accurately measured volume of the diluent. For most photometers 20  $\mu\text{l}$  blood is added to 5 ml of diluent, a 251-fold dilution. The optical density at 540 nm of this solution is directly proportional to the concentration of the pigment. The result should be expressed in terms of grams of hemoglobin per deciliter of blood (g/dl). The use of a scale based on "percent of normal" is discouraged.

Two other methods currently in use depend on visual colorimetry. They are considerably less accurate than the spectrophotometric assay and are used only because of their low cost and the portability of the apparatus. Oxyhemoglobin is the pigment measured in the *Spencer hemoglobinometer*. A hemolysed sample of blood, placed in one half of a glass chamber, is compared by transmitted green light from a battery-lit bulb with a variable standard on a split screen.<sup>310</sup> This permits matching in a color

range to which the human eye is more sensitive, as compared with red. ~

In the *Sahli hemoglobinometer*<sup>310,339</sup> blood is diluted with 0.1 N hydrochloric acid, the hemoglobin of the blood being thus converted to acid hematin which is brown in color. The resulting mixture is then diluted with water until a match with the brown glass standard is attained, and the hemoglobin value is read from the scale by noting the height of the column of the diluted acid hematin.

### Comparison of Methods

The most important characteristics of the various measures of red cell concentration are tabulated in Table 3-5. It is apparent that the error of the red cell count by the hemocytometer method and that of hemoglobin by the Sahli or Spencer methods are great enough to make them unsatisfactory except where cost and portability of equipment are overriding factors.

If a satisfactory specimen of anticoagulated blood can be obtained, the VPRC by the macro method is the most useful, single determination. Not only is it simple and accurate, but with little extra effort additional useful data are available. The microhematocrit is a satisfactory, alternative screening procedure if only limited amounts of blood are available. Its principal disadvantage is that its error increases at low values. Measurement of hemoglobin by the cyanmethemoglobin method is also an excellent screening method. Although the red cell count by electronic methods can be used to detect anemia, its chief application is in the calculation of red cell indices, as described below.

### Indices of Red Cell Size and Hemoglobin Concentration

It was not until more than two centuries after Leeuwenhoek compared the diameter of red corpuscles with that of a grain of sand that further attempts were made to study the size of these cells. Thomas Young, in 1813, and Piiper, more than a century later (1919), showed the value of the principle of diffrac-

tion for the measurement of small objects, including red cells. It was not until the latter half of the 19th century that the diameter of red corpuscles in various diseases was measured and the significance of variations in the size and hemoglobin content of these cells began to be appreciated. In 1864, Welcker demonstrated that the diameter of the red cells in chlorosis is less than normal, and, in 1876, Sorensen pointed out that an increase in the size of the cells is one of the most characteristic features of the blood in pernicious anemia. Hayem introduced the color index in 1878. Malassez described the normal variation in the diameters of red corpuscles, and also calculated the "titre hémoglobique" to micromicrograms. Following the introduction of the hematocrit, Capps introduced the volume index (1903) and made important observations concerning the anemias. Price-Jones' pautostaking measurements of the diameters of red cells, which are responsible for the attention that ultimately came to be paid to cell size, were commenced in 1910. The relationship of hemoglobin to volume of cells attracted Malassez' interest and similar studies were made by Hart (1881) and by Herz and Bonninger (1919). Haden introduced the term "saturation index" (1923). The terms "mean corpuscular volume," "mean corpuscular hemoglobin," and "mean corpuscular hemoglobin concentration" and methods for their calculation were introduced in 1929 by Wintrobe.<sup>351</sup>

#### Determination of Erythrocyte Size

**DIAMETER.** The measurement of cell diameter can be accomplished by measuring the individual cells, by measuring their diffraction patterns, or by the use of an image-splitting microscope eyepiece.

The method used by Price-Jones<sup>342</sup> in his pioneer studies is relatively accurate but laborious. It consisted of the projection of a stained blood film on paper, outlining the cells in pencil, and measurement of the maximum and minimum diameters of each of 500 or 1000 cells. Less time-consuming modifications have been devised.<sup>340,349</sup> A simpler

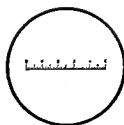


Fig 3 23. Micrometer disk (Courtesy of Carl Zeiss, Inc)

but less accurate method consists in the direct measurement of the cells as seen in the microscope by means of a disk on which a scale has been etched (micrometer disk, Fig. 3-23). The disk is inserted into the eyepiece of the microscope, and the scale must be calibrated in relation to the magnification of the microscope being used. The diameters of the erythrocytes may be recorded graphically to show the number of cells of various sizes. This has come to be known as the "Price-Jones curve" (Fig. 14-4, page 570).

The English workers found that mean cell diameter, as measured by a modification of the Price-Jones method,<sup>350</sup> can be carried out with considerable precision.<sup>304</sup> However, the time required to perform the measurements seriously limits the utility of the procedure.

The *diffraction method* for measuring mean corpuscular diameter depends on the fact that a beam of white light passing through a film containing numerous small particles is diffracted by the edges of these particles to produce a set of colored, concentric circles. With an appropriate optical system the individual spectra can be made to coincide, and from the radius of the yellow circle the mean cell diameter can be calculated. This method was introduced by Pijper in 1919.<sup>341</sup> Since that time, numerous instruments, all based on the same principle, have been described.<sup>302,311,327,328</sup>

Still another way to obtain a size distribution of erythrocyte diameters involves the use of the *Watson image-splitting eyepiece*.<sup>312</sup> With this instrument, manipulation of the control knob separates the cell image into two overlapping images. Rotation of the knob can be continued until the split images separate

far enough to just touch. The degree of movement is recorded and converted to vertical diameter by means of a calibration factor. This technique can be used for rapid screening for cell size or for the construction of a Price-Jones curve.

**VOLUME.** The mean volume of the red corpuscles may be calculated from the erythrocyte count and the VPRC by means of the formula,

Mean corpuscular volume (MCV) =

$$\frac{\text{vol. packed red cells (l/l)} \times 1000}{\text{red cell count} (\times 10^{12}/\text{l})}$$

This formula was designed in order that the result may be expressed in femtoliters (fl;  $10^{-15}$  l).

Example: RBC,  $3.58 \times 10^{12}/\text{l}$ , VPRC, 0.39 l/l

Then,  $\text{MCV} = \frac{390}{3.58} = 109 \text{ fl}$

The electronic cell counter (Chapter 1, page 13) can be employed to record cell volume distribution curves.<sup>308,313 314,324 336,345</sup>

This is done by varying the upper and lower thresholds systematically and recording the differences in counts in these various electronic "windows." To obtain absolute values, the relation of the window number to cell volume must be known. A method also has been described for the determination of frequency distribution curves of red cell volumes by the use of a 100-channel pulse height analyzer.<sup>334</sup> Cell volume distribution curves have also been constructed with photoelectric measurements of the sedimentation of erythrocytes in a dilute suspension of Ringer's solution.<sup>325</sup>

**THICKNESS.** The thickness of red corpuscles, like their diameter, can be measured microscopically as they lie on edge in wet films, as well as by a cellophane section method.<sup>346</sup> The mean thickness may also be estimated from the mean corpuscular volume and the mean diameter, by regarding the cells as short cylinders. Thus,

Mean corpuscular thickness (MCT) =

$$\frac{\text{mean corpuscular volume}}{\pi \left( \frac{\text{mean diameter}}{2} \right)^2}$$

### *The Estimation of the Hemoglobin Content of the Red Cells*

Measurements of the hemoglobin content of the red cells are necessarily indirect and are derived from the blood hemoglobin concentration, the red cell count, and the volume of packed red cells.

The ratio of hemoglobin to red cell count, which indicates the amount of hemoglobin in the average corpuscle, may be expressed in absolute terms.

Mean corpuscular hemoglobin (MCH) =

$$\frac{\text{hemoglobin (g/l)}}{\text{red cell count} (\times 10^{12}/\text{l})}$$

The formula has been calculated so that the result is expressed in picograms (pg; equivalent to  $\mu\text{g}$  or  $10^{-12}$  g).

Example. RBC,  $3.6 \times 10^{12}/\text{l}$ , hemoglobin, 13.6 g/dl

Then, mean corpuscular hemoglobin is  $\frac{136}{3.6} = 38 \text{ pg}$

Another important ratio, as will be shown in the chapters on the anemias, is that of hemoglobin to volume of packed red cells. This measures the concentration of hemoglobin in the average red corpuscle.

Mean corpuscular hemoglobin concentration (MCHC) =

$$\frac{\text{hemoglobin (g/dl)}}{\text{vol packed red cells (l/l)}}$$

The result is expressed in grams of hemoglobin per decaliter of packed red cells (g/dl).

Example: Hemoglobin, 13.6 g/dl, vol packed red cells, 0.39 l/l of blood

Then, mean corpuscular hemoglobin concentration

$$= \frac{13.6}{0.39} = 35 \text{ g/dl}$$

With the aid of the nomogram shown in Figure 3-24 or by the use of a slide rule<sup>203,310</sup> the erythrocyte indices can be calculated easily.

The difference between mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) should be clearly understood. The former measures the weight of hemoglobin in the average red



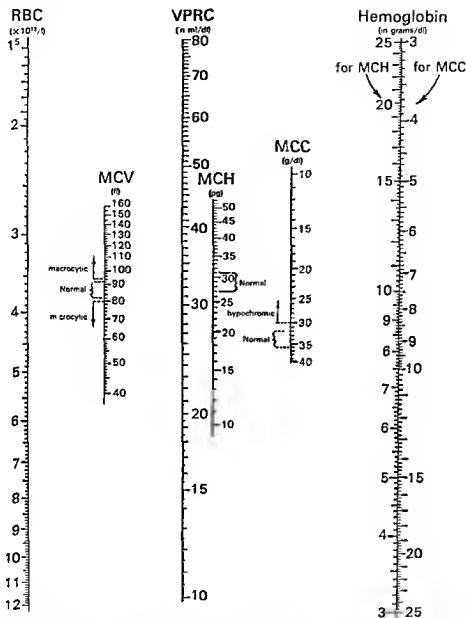


Fig 3-24 Nomographic alignment chart for reading of red cell indices. For mean corpuscular volume (MCV) join the value for red cell count and volume of packed cells (VPRC) by means of a ruler, preferably a transparent one (A very satisfactory one is prepared by scratching a straight line on a strip of clear x-ray film and filling it in with ink). The reading is made where the line intersects MCV. Similarly, where a line joining RBC and hemoglobin intersects MCH the reading for mean corpuscular hemoglobin is made, where a line joining hemoglobin and VPRC intersects MCC the reading for mean corpuscular hemoglobin concentration is made. Note that the left side of the hemoglobin scale is used for MCH, the right side for MCC (Prepared by Dr Robert E. Mason.)

corpuscle and expresses the results in parts of a gram (picograms). The latter (MCHC) indicates the *concentration* of hemoglobin in the average red corpuscle, ie, the ratio of weight of hemoglobin to the volume in which it is contained, and the result is expressed in

g/dl. The distinction is an important one. In the *normochromic* anemias, increases or decreases in the average size of the red corpuscles (MCV) are associated with corresponding increases or decreases in the weight of hemoglobin (MCH) carried in the corpus-

cles, but the concentration of hemoglobin (MCHC) remains normal. In the *hypochromic* anemias (Chapter 16), however, the reduction in the weight of hemoglobin in the average corpuscle is even greater than the decrease in the average cell size; thus, the MCHC is subnormal.

Except in hereditary spherocytosis (page 753), and sometimes in homozygous sickle-cell and Hgb C diseases, the MCHC does not exceed 37 g/dl. Thus, the term *hyperchromic* is rarely appropriate. The value of 37 g/dl is near the upper limits of solubility of hemoglobin and further concentration might be expected to result in crystallization.

Now outmoded is the *color index*, which expresses the average amount of hemoglobin in the red cell in relation to arbitrarily chosen normal values for hemoglobin and red cell count. It was a misunderstanding as to the meaning of this index that led to a mistaken impression regarding the hemoglobin concentration in the red cells in pernicious anemia. Because the color index is increased in this disease it was incorrectly assumed that the red corpuscles in this condition were "hyperchromic" or supersaturated with hemoglobin. It is true that the amount of hemoglobin in the average cell, as measured by MCH or by color index, is increased, but only in proportion to the increase in the average size of the cells. The MCHC is not increased. The darker than normal appearance of these corpuscles when examined under the microscope, and the lack of the normal central pallor, are due to their increased thickness.

### Reproducibility of the Red Cell Indices

The MCV, MCH, and MCHC are sometimes referred to collectively as the red cell indices or the corpuscular constants. The former term is to be preferred since these measures are not "constants" in the usual sense of the word. With skilled technicians using the preferred methods, the red cell indices can be determined with a high degree of precision (Table 3-6). Obviously, the use of less precise or less accurate methods increases the error of these indices.

It must be emphasized that the degree to which the values are to be relied upon relates not only to the methods employed, but also to the skill and care with which they are performed. Undoubtedly, in all instances, whether the technique is beyond criticism or where it is only mediocre, the examination of the blood smear by the physician himself is extremely important. In this way, gross errors are not likely to pass unnoticed and a visual picture of the morphologic appearance of the blood can be obtained; in addition, sometimes important information may have been overlooked by persons less directly interested in the patient.

### Staining and Enumeration of Reticulocytes

New Methylene Blue N (Color Index 52030) is superior to the classic brilliant cresyl blue because of its uniform performance and the sharp, blue staining of the reticulum.<sup>307,317</sup> In making the reticulocyte count, all red corpuscles that contain blue staining threads or granules are counted. In relatively mature reticulocytes, only a few blue granules or scattered threads will be found, but these should still be classed as reticulocytes. The percentage among 500 or, better still, 1000 red corpuscles is counted. An accurate estimate can be made only if the red corpuscles are distributed evenly, without overlapping

Table 3-6. Reproducibility of the Red Cell Indices\*

Index	Method Used for RBC	Error (%) ( $\pm 2$ CV)
MCV	Hemocytometer	9.5
	Electronic	3.2
MCH	Hemocytometer	10.0
	Electronic	3.6
MCHC		1.5

\*The VPRC was determined by the macro method and the hemoglobin by the cyanomethemoglobin method.

CV = coefficient of variation

From Cartwright,<sup>310</sup> courtesy of the author and Grune & Stratton

clumps or rouleaux. There should be no visible conglomeration at the free edges.<sup>375</sup>

The margin of error in reticulocyte counting is great. It is greatest when counts are made on different preparations by different observers and is large even when they are done on the same preparation by different observers. The range of values may be as great as 100% above or below the mean value. Repeated reticulocyte counts by the same observer on the same specimen of blood vary less. It is desirable, therefore, that the same observer make all the counts on any one patient. Reticulocytes may be expressed as percent of red cells, or this figure may be multiplied by the red cell count to yield an "absolute number" (reticulocytes  $\times 10^9/l$ ). Methods for "correcting" the reticulocyte count for anemia and for premature release ("shift") are discussed in Chapter 20 (page 731).

Techniques for determining the "absolute number" of reticulocytes with the hemocytometer<sup>122</sup> have not been very satisfactory because, at the magnification that can be used with the ordinary objective, reticulocytes cannot be made out readily. Björkman,<sup>305</sup> however, introduced a method whereby reticulocytes are first stained in a capillary pipet, and afterwards are fixed in a diluent consisting of 0.30% potassium thiocyanate (KSCN) in 0.05 normal sulfuric acid. The fixation process is attended by an escape of hemoglobin from the red cells which allows them to show up more clearly than otherwise.

The speed and accuracy of reticulocyte counts are increased in a method that takes advantage of the fact that the reticulum is composed largely of RNA. Acridine orange combines with RNA and the resulting complex is seen as an orange-red fluorescence when exposed to ultraviolet light.<sup>317</sup>

### The Total Quantity of Blood

A variety of physiologic mechanisms operate to maintain blood volume within narrow limits. Therefore, in most clinical situations the total quantity of erythrocytes in the body tends to be closely related to their concen-

tration in the blood. There are, however, well-known clinical entities in which the blood volume may deviate considerably from normal. These include hemorrhage, dehydration, overhydration, congestive heart failure, renal disease, cirrhosis of the liver, and others. In these disorders the measures of concentration may not accurately reflect total quantity. Measurements of red cell volume, the plasma volume, or both may be necessary if the true clinical situation is to be assessed.

### Methods for Determination

The direct method of blood volume estimation was employed in 1854 by Welcker who bled animals to death, washed out the vessels with water, and extracted the hemoglobin still remaining in the tissues by mincing the organs and placing them in water for several days after the bile, intestinal contents, and urine had been removed.<sup>395</sup> Welcker concluded that the blood volume of mammals constitutes 7.7% of the body weight. This value was confirmed in man by Bischoff whose subjects were two condemned criminals.<sup>395</sup>

Most modern methods depend on the dilution principle. A substance is introduced into the circulation, and, after an appropriate interval for mixing, the space in which it is distributed is calculated from the degree to which it is diluted. Thus,

$$\text{Distribution space} = \frac{\text{amount injected}}{\text{concentration}}$$

If the substance is confined to the plasma, then the distribution space is equivalent to plasma volume; if confined to the erythrocytes, the distribution space becomes a measure of red cell volume.

With an ideal label, the distribution space would correspond precisely to the circulation volume to be measured, and excretion, destruction, or loss to tissues would be negligible, or at least could be quantitated. Furthermore, the label should mix quickly and completely with various parts of the circulation, and should be free of significant side effects. Finally, methods of measuring the

label should be accurate, convenient, and inexpensive.

Over the years, many different labels have been used in attempts to achieve these objectives. These include isotonic sodium chloride solutions,<sup>377</sup> tetanus antitoxin,<sup>372</sup> aca-cia,<sup>390,391</sup> and homologous precipitating anti-serum.<sup>379</sup> The use of carbon monoxide as a label for hemoglobin, introduced in 1882 and improved in recent years,<sup>373</sup> was shown to give significantly higher (16%) values than are obtained by other methods<sup>393</sup> because hemoglobin in bone marrow, muscles, and elsewhere, and other extravascular pigments, are tagged, in addition to the hemoglobin in the circulation. Subsequently, the introduction of the dye dilution method<sup>389</sup> provided a simple technique that could be applied widely. The introduction of radioactive elements in physiologic studies led to a geometric acceleration of investigations in this field. The first result was an accumulation of discordant values. Now, however, many of the problems involved in the measurement of blood volume are understood, even if not completely solved, and one can define precautions that must be taken, as well as the values that can be obtained under a variety of circumstances.<sup>384</sup>

**PLASMA VOLUME.** The introduction of the nontoxic, slowly diffusing blue dye, T-1824 ("Evans blue"), solved some of the problems presented by the original "brilliant vital red." The use of T-1824 involves the injection of a known quantity of dye and the subsequent withdrawal of samples of blood at appropriate intervals for determination of dye dilution. The dye is firmly bound to the plasma albumin<sup>385</sup> and therefore is diluted in the initial distribution space of albumin, which is approximately equivalent to the plasma volume.

Special precautions must be taken to determine the exact amount of dye injected and that present in the samples.<sup>384</sup> Methods have been devised for extraction of the dye, thus permitting greater accuracy in the measurement.<sup>373</sup> Graphic analysis of the time-concentration curve on a semi-log plot and

back extrapolation of the disappearance curve provide the accepted means of arriving at the value for the initial concentration of dye needed for calculating the plasma volume.

*Serum albumin* tagged with radioactive iodine ("RISA")<sup>378,384,388,397</sup> gives results comparable with the T-1824 method<sup>396</sup> and, because the half-life of <sup>131</sup>I is only eight days, determinations can be repeated at intervals. Apparatus has been designed to facilitate such repeated determinations (Volumetron).<sup>399</sup> The RISA method is subject to errors similar to those of the dye method but also has certain advantages.<sup>396</sup> It has been observed that albumin may be lost to the vascular bed early in the measuring process, and that this phenomenon is particularly striking in individuals who are ill as opposed to healthy subjects.<sup>398</sup> When albumin is lost in the vascular bed, the initial albumin space will be larger than the plasma volume. A method for compensating for this error has been developed.<sup>398</sup>

Another isotope, <sup>113m</sup>indium, may be useful for plasma volume determinations.<sup>400</sup> Indium binds firmly to transferrin (page 158), and the initial transferrin distribution space, like the albumin space, is roughly equivalent to the plasma volume. Values for plasma volume determined with indium were about 5% higher than those determined with RISA. Since <sup>113m</sup>indium has a short half-life (100 minutes), determinations may be repeated frequently; furthermore, the radiation dose is very low.

**RED CELL VOLUME.** The *Ashby technique* of differential agglutination of red cells (page 197) has been used for the estimation of red cell volume and can be performed with considerable accuracy and without special equipment; however, because the procedure is tedious and scrupulous care is required,<sup>371</sup> it is rarely used. Isotopic methods which include the use of <sup>59</sup>Fe, <sup>32</sup>P and <sup>51</sup>Cr are preferred.<sup>381</sup>

The *radioactive iron* method involves the administration of <sup>59</sup>Fe to a donor, to allow incorporation of the iron into his red corpuscles, and the subsequent transfusion of the

donor's blood into the subject.<sup>381,386</sup> The dilution of the transfused blood is then determined by measuring the radioactivity of the recipient's blood. This cumbersome method is now rarely used.

A simpler technique consists in the introduction of *radioactive phosphorus* ( $^{32}\text{P}$ ) into the erythrocytes *in vitro* and their subsequent return to the circulation, where their dilution is measured.<sup>375</sup> Thus, no problem of securing a donor and no concern regarding blood types arises. Many improvements on the original technique have been introduced.<sup>392</sup>

*Radioactive chromium* ( $^{51}\text{Cr}$ ) has become the most widely used label for measurement of red cell volume<sup>383,394</sup> because it is held more avidly by red cells than is radioactive phosphorus.<sup>394</sup> The amount commonly used is well below the safe radiation dosage for humans. Since there is no significant loss of  $^{51}\text{Cr}$  to the plasma for 24 hours or more, only one or two samples need be taken at whatever postinjection time is considered necessary for complete mixing in the circulation.

**TOTAL BLOOD VOLUME.** Methods are available whereby simultaneous measurements of plasma volume, by means of RISA, and of red cell volume, with  $^{51}\text{Cr}$ , can be made.<sup>380 382 387 401</sup> More often, the total blood volume is calculated from either the plasma volume or the red cell volume and a "corrected" value is determined for the VPRC. It has been shown that the "venous" hematocrit overestimates the proportion of red cells in the circulating blood as a whole, the so-called "body" hematocrit. The VPRC in the capillary bed is substantially lower than in venous blood so that the "body" hematocrit is approximately 91% of the VPRC in venous blood.<sup>376</sup> Under most conditions the ratio of "venous" to "body" hematocrit is a constant one,<sup>386</sup> so that a correction factor of 0.91 can be used. However, in patients with splenomegaly the body hematocrit/venous hematocrit ratio (BH/VH) is increased in direct proportion to the degree of splenic enlargement, because of the increased concentration of erythrocytes in the spleen.<sup>380</sup> In such patients, the increased concentration of

red cells in the spleen offsets the relatively low concentration in small blood vessels so that BH/VH is closer to 1.0 than to 0.91. In the presence of extravascular fluid retention, the ratio may similarly depart from the usual one in conditions of prolonged oligemic shock in which capillary blood flow is altered radically, as well as in patients with congestive cardiac failure.<sup>376</sup>

In addition, the VPRC must be corrected for plasma trapped in the red cell column (page 114).

### The Specific Gravity of Blood

The specific gravity of blood, that is, the ratio of the weight of the blood to the weight of the same volume of water at a temperature of 4° C, may be determined directly by weighing a measured volume of blood. Schmaltz<sup>434</sup> described a capillary pycnometer designed to make this determination. Such a method, however, is time-consuming, and various indirect methods for measuring specific gravity have been devised.<sup>426,427,428 430</sup>

The normal specific gravity of blood as determined by the pycnometric method is given as 1.048 to 1.066 with reported averages of 1.052 to 1.063. It is slightly higher in men (1.057) than in women (1.053). There is a normal diurnal variation of about 0.003, the specific gravity being generally lower in the afternoon and after meals and higher after exercise and during the night.<sup>433</sup> The specific gravity of the blood serum is 1.026 to 1.031, while that of the erythrocytes is 1.092 to 1.095.<sup>430</sup>

The specific gravity of the blood depends on a number of factors, particularly the quantity and hemoglobin content of the red corpuscles and the protein content of the plasma. Since these are usually measured independently, few have been interested in studying the specific gravity of blood in various disease conditions.<sup>433,435,436</sup>

The *copper sulfate method*<sup>431</sup> for measuring specific gravity has a number of advantages as compared with the methods previously used. No precision instruments are required.

The procedure consists in letting drops of plasma or whole blood fall into a graded series of solutions of copper sulfate of known specific gravity and noting whether the drops rise or fall in the solutions. Each drop on entering the solution becomes encased in a sack of copper proteinate and remains as a discrete drop without change of gravity for 15 or 20 seconds, during which its rise or fall reveals its gravity relative to that of the solution. The method is capable of measuring gravities to  $\pm 0.00005$ , the degree of accuracy depending on the number of standard copper sulfate solutions used.

The copper sulfate method still enjoys some popularity as an indirect way of detecting anemia in situations requiring mass screening, for example, of potential blood donors. Under these circumstances, usually only one or two copper sulfate solutions are used, with a specific gravity of 1.055 for men and 1.053 for women. Donors are considered suitable if their blood specific gravity exceeds that of the standard solution.

The microhematocrit method of determining the VPRC (page 113) and electronic methods of measuring "hematocrit"<sup>429</sup> are nearly as rapid as the copper sulfate screening method. Furthermore, in these methods, in addition to deviation from normal, the degree of anemia is estimated. As a result, the copper sulfate method may soon become obsolete in laboratory settings. It has special advantages under battlefield conditions, however, because no power source is required.

### The Blood Viscosity

The simplest method for the measurement of the blood viscosity is by means of a capillary viscosimeter.<sup>437</sup> The instrument is based on Poiseuille's law, namely, that the flow rate of fluids in capillaries of equal caliber, under the same pressure and at the same temperature, depends upon the inner friction (viscosity). In the viscosimeter, blood and, in another tube, distilled water are drawn under equal pressure through capillary tubes, of equal caliber and length, into graduated tubes. The blood is forced to reach a certain

point. The volume of distilled water that has been drawn up under this pressure is indicated by the graduated tube containing the water. Since volume is inversely proportional to viscosity, the reading on the water tube indicates the viscosity of the blood in relation to that of distilled water. The test requires only one drop of blood and may be carried out in 30 seconds.

The relative viscosity of the blood of healthy adults ranges from 3.5 to 5.4 (average 4.5). That of serum is 1.4 to 1.8; values for plasma are about 20% higher.

The viscosity of blood as measured in capillary viscosimeters is not the same as it is within the vasculature.<sup>446</sup> Blood is not a Newtonian fluid and, therefore, does not obey Poiseuille's law. As a result, blood viscosity will have many different values in different parts of the vascular system. In general, within vessels such as the ascending aorta, where the "shear rate" (a function of vessel diameter and flow rate) is relatively great, viscosity will be low; increasing values are expected as vessel size and flow rate decrease. An exception to this generalization exists with respect to capillaries, in which vessel diameter is only slightly greater than cell size. The viscosity, under such circumstances, is related only to plasma viscosity, rather than to whole blood viscosity.<sup>446</sup>

At a given shear rate, blood viscosity is related to the composition of the blood, especially the quantity of erythrocytes, the concentration of macroglobulins, and, in some instances, the number of leukocytes. The influence of the VPRC on viscosity for two different vessel sizes is shown in Figure 3-25. In general, there is a sharp increase in viscosity with increases in VPRC above the normal range.

The influence of macroglobulins on blood viscosity is indicated in Figure 3-26.<sup>440</sup> Although levels of most of the other immunoglobulins have only modest effects on viscosity, those belonging to the class IgG<sub>3</sub> polymerize readily and lead to changes similar to those found in macroglobulinemia.<sup>439</sup> Symptoms of increased blood viscosity due to macroglobulinemia include a tendency to

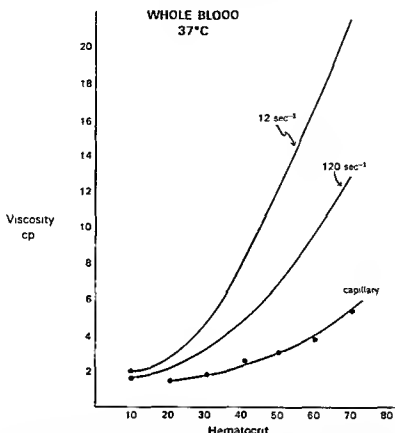


Fig 3-25 Relation of VPRC (hematocrit) to blood viscosity in centipoise (cp) as measured in a capillary viscosimeter compared with that calculated for shear rates of 120 sec<sup>-1</sup> (ascending aorta) and 12 sec<sup>-1</sup> (medium arteriole) (From Wells and Merrill<sup>440</sup> courtesy of the authors and American Journal of Medicine)

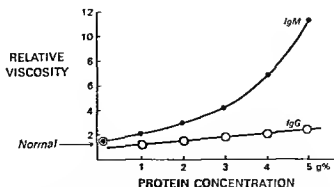


Fig 3-26 Relation of macroglobulin (IgM) and IgG concentrations to viscosity (From Fahey et al<sup>440</sup> courtesy of the authors and the Journal of the American Medical Association)

bleed from the nose and gums, ocular disorders, and neurologic signs.<sup>439,440</sup> In polycythemia, similar symptoms may also be related to excessively viscous blood.

The quantity of leukocytes is of significance only when they are greatly increased in number. This is true particularly in myelocytic leukemia because the myeloid leukocytes are larger than lymphocytes. The increased blood viscosity in leukemia is probably responsible for some of the symptoms of this disease, such as dizziness, roaring sensations in the ears, and perhaps even priapism. Stephens<sup>444</sup> observed relative blood viscosities greater than 9 in patients with chronic myelocytic leukemia and demonstrated that blood circulation time was greatly delayed. The influence of blood platelets on viscosity is not clear.<sup>441</sup>

An increase of carbon dioxide in the blood is said to increase viscosity through changes in osmotic relationships. Venous blood has been found to possess a higher viscosity than arterial blood.

Naegeli<sup>443</sup> considered viscosimetry to be an important adjunct in routine blood examinations in the sense that a change in many factors is at once reflected by alterations in blood viscosity. In Naegeli's clinic, charts were devised whereby, from the viscosity of the plasma and whole blood, the protein content of the plasma and the volume of packed red cells could be calculated. Viscosimetry received considerable attention in European clinics but has attracted only limited attention in the United States.<sup>448</sup>

### Suspension Stability of the Blood—The Sedimentation Test

The blood is essentially a suspension of corpuscles in plasma. In 1918, Fahraeus,<sup>462</sup> while seeking an early test for pregnancy, discovered that the suspension stability of the blood is altered in pregnancy, but he found that the speed of sedimentation is also accelerated in many diseases. A test was thus discovered which, because of its simplicity and its wide applicability, soon became very popular.

Variations in the suspension stability of the blood probably led to the development of the theory of the four humors of the ancient Greeks. When blood is withdrawn from a healthy person, it clots quickly and two portions, the clot and the serum, are formed. In the presence of disease, sedimentation of the red corpuscles may be so accelerated that some of the corpuscles quickly settle to the bottom of the vessel in which the blood has been collected and, since they are deprived of oxygen, appear very dark. Above this, the corpuscles still containing oxyhemoglobin, and therefore appearing red, will be found. The rapid sedimentation of the erythrocytes permits some separation of the leukocytes and these, especially when there is leukocytosis, form, together with fibrin, a well-defined grayish-white layer in the uppermost portions of the clot. These three portions of the blood were named respectively, "melancholia" or "black bile," "sanguis" or "true blood," and "phlegma" or "mucus." The blood serum itself formed the fourth humor, "cholera" or "yellow bile." Ill health was attributed to the failure of the four humors to mix. Not until the time of Paracelsus, it seems, was it considered possible that this separation into several layers might be the result, rather than the cause, of disease. The grayish-white layer of fibrin and leukocytes continued for centuries to occupy the attention of physicians, being referred to as "crusta inflammatoria," "buffy coat," or "size." Venesection was practiced in order to rid the body of this supposedly noxious substance.

### The Nature of the Sedimentation Phenomenon

The rate of erythrocyte sedimentation depends upon the interaction between opposing physical forces (Fig. 3-27). Settling occurs because the density of the red corpuscles is greater than the density of the medium. The fall of the red corpuscles causes an upward displacement of the medium, thus producing an upward current and a retarding force. In blood drawn from normal persons





Fig 3-27. The effect of certain factors on sedimentation rate. Arrows pointing downwards represent the downward force of the red corpuscles (DF). Arrows pointing upwards represent the retarding effect (RF) of the medium and the limitation produced by the end of the tube.

Normal blood  $RF \text{ almost} = DF$

Anemia  $RF < DF$

Less retardation from displacement of plasma by red corpuscles

Inclined tube  $RF < DF$  Plasma streams along upper side of tube under the glass, exerting no retarding effect

Increased rouleau formation  $RF < DF$  because  $RF$  depends on relative surface. The larger the volume, the smaller the relative surface.

the concentration of the red corpuscles is relatively great, and a relatively large volume of plasma must be displaced upward if much sedimentation is to occur. Actually the downward and upward forces in normal blood are nearly equal and consequently little settling occurs.

When the number of red corpuscles is less than normal, there is less retardation of sedimentation by the red corpuscles themselves. No matter how high the column of blood may be, its length is not infinite and retardation is produced by the cells striking the bottom and piling up on one another. Obviously, the fewer the cells the less this effect will be. Methods have been described for "correcting" for the effect of anemia.<sup>477,483</sup> These are discussed more fully below.

The statements that have been made refer to a column of blood that stands in the vertical position. Any deviation from the vertical causes an acceleration in the rate of sedimentation. This occurs because the plasma streams along the upper side of the tube from

which the red corpuscles have already settled out and the red corpuscles therefore encounter less hindrance from displacement of the medium.

The factor that is of chief importance in affecting the sedimentation rate in disease is the size of the sedimenting particle. The larger the volume of the particle, the smaller is the relative surface. The upward or retarding force is a function of the surface area exposed to the medium. The downward force depends on the weight of the particle. In the presence of certain conditions the aggregation of the red corpuscles (rouleaux formation) is greatly increased, and this results in the production of corpuscular aggregates of large volume but relatively small surface area. This phenomenon is the chief cause of the acceleration of sedimentation rate encountered in the presence of disease and in pregnancy.

The reason for the increased aggregation is not entirely clear; however, it probably results from changes in the negative surface charge (zeta potential) of the red corpuscles.

This charge, a function of the sialic acid groups on the cell membrane (page 100), can be attenuated by the dielectric effect of proteins in the surrounding plasma, especially asymmetric macromolecules like fibrinogen and gamma globulin.<sup>455</sup> Thus, alterations in sedimentation rate generally reflect alteration in these plasma proteins. Indeed, the addition of fibrinogen leads to a greatly increased sedimentation rate,<sup>467</sup> and the fact that the plasma fibrinogen is usually increased when the sedimentation rate is accelerated led to the conclusion that increased sedimentation velocity usually is the result of an increase in the quantity of fibrinogen in the plasma.<sup>465,473</sup> The addition of other protein fractions to blood also leads to an acceleration of sedimentation,<sup>462</sup> but the effect is less marked than that produced by the addition of fibrinogen. Close correlation between plasma globulin and sedimentation rate has been observed,<sup>452,467</sup> particularly with plasma proteins of high molecular weight, such as the  $\alpha_2$ - and  $\gamma_1$ -macroglobulins.<sup>470</sup> Much less commonly, an increase in sedimentation rate may be the result of erythrocyte factors.<sup>468,480</sup>

A method for measuring the sedimentation rate was devised in 1924 by Westergren,<sup>481</sup> and continues to be popular. It requires a special tube 30 cm in length, open at both ends, and a rack to fix the tubes in a vertical position during sedimentation. Also widely used is a method in which sedimentation is measured in the Wintrobe hematocrit.<sup>483</sup> With both methods, results are expressed in millimeters per hour (for normal values, see Appendix A). The results with the two methods do not always correspond, chiefly because the Westergren tube is longer, and sedimentation in it is less retarded by packing of cells. The Wintrobe method has the important advantage of allowing the subsequent determination of the VPRC, the volume of packed leukocytes and platelets, and the icterus index in the same tube and with the same volume of blood used for the sedimentation rate. It is often preferred for these reasons.

A number of technical variations have been introduced,<sup>463</sup> including micro methods,

<sup>460,472,478</sup> sedimentation at an angle of 45 degrees to accelerate the process,<sup>457,479</sup> and improvements in the Westergren method.<sup>461,464</sup> Most sophisticated of the improved methods is the "zeta sedimentation ratio" (ZSR), a measure of the degree of packing of erythrocytes occurring during four, 45-second cycles of dispersion and compaction in special capillary tubes.<sup>455</sup> This measure is linearly related to increases in fibrinogen or gamma globulin, can be performed on micro quantities, and is not affected by anemia. It is faster than either the Westergren or Wintrobe methods, but requires a special instrument, the Zetafuge.

The values are not expressed in millimeters per hour but as ml/dl (vol %) and the normal range is 40 to 51 ml/dl in both males and females.

With either the Wintrobe or Westergren technique, values are affected by the VPRC,<sup>460,483</sup> and various methods for "correction" for anemia have been devised.<sup>453,469,483</sup> Such corrections do not apply to all forms of anemia, however, because differences in the size<sup>475</sup> and hemoglobin content<sup>476</sup> of the red cells also affect the sedimentation rate. Furthermore, anisocytosis may interfere with rouleau formation, and poikilocytosis such as that encountered in sickle cell anemia actually prevents the formation of rouleaux. In sickle cell anemia, sedimentation is very slow even when there is marked anemia.<sup>456</sup> An important objection to correction for anemia is the fact that all methods of correction are only approximate and are also artificial. Furthermore, many who use such corrections do not recognize their artificiality and tend to make more of this ultra-refinement than it merits. It seems best, therefore, to abandon correction of sedimentation rate for anemia; however, it is wise to record simultaneously the VPRC, thereby giving an indication of the presence or absence of anemia and its degree.

### Variations in Disease

The sedimentation test is a nonspecific reaction which may be compared with the body temperature, the pulse rate, and the

leukocyte count, in that it gives information of a general character. It is a measure of the presence and intensity of morbid processes within the body. The test is a useful supplement to clinical methods because it may be accelerated when the temperature, pulse, and even the leukocyte count are normal, particularly in chronic disorders and in localized inflammatory diseases.

In general, sedimentation rate is increased in all acute general infections while, in localized acute inflammatory conditions, variations in sedimentation rate depend on the nature and severity of the morbid process. Thus, in localized acute suppurations, such as pelvic inflammatory disease, there may be a pronounced acceleration even when the pulse rate and temperature are normal. Again, in chronic localized infections the rate varies with the extent and nature of the infection. Uncomplicated neoplasms are not necessarily associated with rapid sedimentation even when malignant.<sup>474</sup> If the neoplasm is malignant, sedimentation tends to be accelerated when the tumor is very vascular, when there is a tendency to break down, or when there is much reaction about the tumor.<sup>471</sup>

It has been reported that an increased sedimentation rate due to inflammation can be distinguished from that due to neoplastic disease. The method consists of performing the test before and after incubation of the plasma for four hours at 37° C. Such incubation is said to reduce the sedimentation rate considerably less in neoplastic disease than in inflammatory disease.<sup>466</sup>

One of the most important uses of the sedimentation test is in calling attention to the presence of more or less occult disease and for this reason it is as valuable a routine procedure in examination as is urinalysis or the estimation of blood pressure. Not infrequently the sedimentation rate may be accelerated when clinical and other laboratory studies yield negative results. If technical error can be ruled out, such a finding should be considered a challenge to the acumen of the physician, and a diligent search must be made for its cause. One cannot insist that an accelerated sedimentation rate always indi-

cates the presence of pregnancy or disease,<sup>451</sup> especially in elderly patients.<sup>451</sup> However, in clinical practice it may well be regarded as a sign of disease until the physician is thoroughly satisfied that the patient is perfectly well. On the other hand, a normal sedimentation rate does not necessarily mean that all is well. Normal rates occasionally have been found in patients with neoplastic conditions of the liver, cirrhosis,<sup>458</sup> chronic passive congestion, cachexia, or other serious disease.<sup>482</sup>

As an aid in differential diagnosis, the sedimentation test may be useful. Other things being equal, an accelerated rate suggests organic disease rather than a functional disorder.

Serial measurements of the sedimentation rate may serve as a guide to the progress of disease that has already been recognized. This is particularly true in regard to pulmonary tuberculosis,<sup>459</sup> rheumatic carditis,<sup>438</sup> rheumatoid arthritis, and certain malignancies, including Hodgkin's disease.

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## *Production of Erythrocytes*

### **Nutritional Requirements for Red Cell Production**

- Protein and Amino Acids
- Vitamins
- Minerals
- Iron Metabolism
  - Total Body Iron
  - Iron Balance
  - The Iron Cycle
- Hemoglobin Structure and Synthesis
  - Heme
  - Globin
- Control of Erythropoiesis
  - Tissue Oxygen
  - Erythropoietin
  - Role of the Nervous System
  - Other Erythropoietic Substances

### **Nutritional Requirements for Red Cell Production**

From what is known about the composition of the red cell, it is apparent that a great variety of materials is required for erythropoiesis. None of these is unique in the sense that it is needed for erythropoiesis alone and not for other systems of the body as well. Anemia is a late sign of nutritional deficiency. The effects of deficiency develop insidiously and are widespread, but most other tissues are less readily available for examination than are the cells of the blood. Consequently, examination of the blood constitutes an important means for the detection of nutritional

deficiency. In addition, in relation to certain essential substances a system of priorities seems to operate, resulting in deprivation of the erythropoietic system before functional disturbances in other tissues are detected. Thus, when iron is in limited supply, hemoglobin production is impaired before deficiencies in certain other iron enzymes are detectable (page 659).

For consideration in this section are those nutrients for which requirement is so critical that their lack results in detectable changes in the blood. Lack of the many other required substances results in critical changes in other tissues long before the hematopoietic system is seriously affected.

### **Protein and Amino Acids**

Knowledge concerning the substances needed for hematopoiesis was first gained by observing the effects of dietary restrictions. Whipple and his associates pioneered in the field by showing clearly the importance of protein in erythropoiesis.<sup>10,17</sup>

Dietary deprivation of protein leads to anemia in a variety of experimental animals, including pigs,<sup>4</sup> rats,<sup>10</sup> and monkeys.<sup>7,11</sup> The anemia appears to result chiefly from impaired erythrocyte production since reduced circulating reticulocytes, reduced iron utilization by the red cell, and erythroid hypoplasia of the marrow are observed. However, there may also be a reduction in the red cells' life span, perhaps because of structural defects in these cells.<sup>6</sup>

That protein deficiency in man leads to anemia may be inferred from observations of prisoners of war<sup>8,15</sup> and patients with kwashiorkor, a syndrome of severe protein-calorie malnutrition.<sup>1,7,11,12</sup> The anemia in kwashiorkor is complex since there may be deficiencies in many nutrients besides protein, as well as complicating infections. Nevertheless, in most instances the anemia has responded to administration of protein in the form of cow's milk without other added nutrients.<sup>1,11</sup>

In rats, the anemia of protein deficiency can be prevented or alleviated by the administration of erythropoietin.<sup>10</sup> This observation suggests that the anemia of protein deprivation does not result from a fundamental lack of hemoglobin substrates at the oörmoblast level. Instead, when the organism is confronted with a limited supply of amino acids, the erythropoietin control mechanism is altered. This results in a diversion of amino acids to the synthesis of other proteins, perhaps ones that are more urgently needed.

With more severe or prolonged protein starvation, it is possible that other mechanisms play a role in the development of anemia, such as diminished production of hormones, especially thyroxine, adrenocorticoids, and testosterone.<sup>2</sup>

All of the ten "essential" amino acids are required for hematopoiesis in the rat. In the order of decreasing importance these are: histidine, valine, leucine, isoleucine, lysine, arginine, methionine, tryptophan, phenylalanine, and threonine.<sup>13</sup> Glycine also is required. Peptides are used less efficiently than are simple amino acids. In dogs made anemic by repeated bleeding, proline, threonine, glutamic acid, cystine, aspartic acid, histidine, glycine, phenylalanine, methionine, tryptophan, leucine, tyrosine, lysine, valine, isoleucine, alanine, arginine, and hydroxyproline, when given in amounts of 1 to 2 g daily, were found to be effective, in decreasing degree, in producing 34 to 10 g hemoglobin over a two-week period.<sup>17</sup>

The specific role of each amino acid in blood formation is yet to be determined. There is no correlation between the quantity

of an amino acid found in globin (page 174) and its effectiveness in hemoglobin regeneration.<sup>3</sup> The feeding of a diet containing acid hydrolyzed casein results in the development of a moderate anemia in rats and severe normocytic or slightly microcytic anemia, without hypochromia, in swine.<sup>5</sup> These anemias are accompanied by leukopenia, hypoplastic or normal bone marrow, and a normal serum iron level and are in all likelihood due to tryptophan deficiency. The severe anemia that develops in rats maintained on deaminized casein appears to be due to the effects of hexahomoserine, which acts as a lysine antagonist.<sup>9</sup>

## Vitamins

### Vitamin B<sub>12</sub> and Folic Acid

The standard salmon-bread ration that Whipple and coworkers fed dogs made anemic by bleeding<sup>17</sup> was deficient in a number of respects. No doubt some of the beneficial effects of the foods tested in their experiments were due to the iron and the vitamins that were contained therein. The anemia, leukopenia, and granulocytopenia produced experimentally in monkeys by feeding them a diet comparable to that taken by natives in India,<sup>179</sup> as well as the "tropical macrocytic anemia" of these natives, were cured by giving autolyzed yeast, a recognized source of "vitamin B." Brewer's yeast was also shown to be effective in the treatment of pernicious anemia if given in large quantities,<sup>180</sup> a response that subsequently was recognized to be due to the yeast's folate content. With the isolation of vitamin B<sub>12</sub> from liver<sup>146,158</sup> it could be demonstrated that this substance is the same as the "extrinsic factor" that Castle postulated as requiring "intrinsic factor" for its absorption (page 138), and that it is also identical with the "antipernicious anemia principle" that was thought to be formed through the interaction of the "intrinsic" and "extrinsic" factors. It was thus indicated that deficiencies of vitamin B<sub>12</sub> and of folic acid are involved in the development of megaloblastic anemia in man.

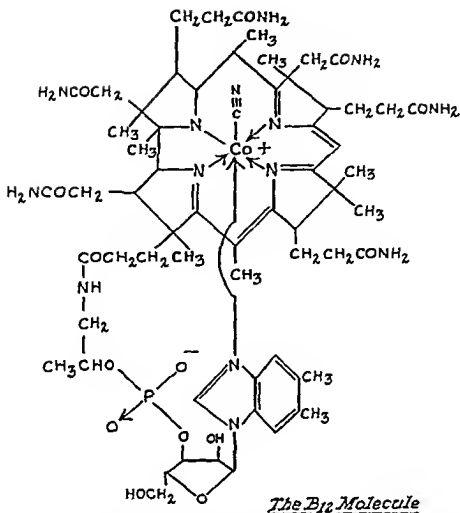


Fig 4-1. Structure of cyanocobalamin, the official form of vitamin B<sub>12</sub>. There are six primary amide groups and one secondary amide joining the aminopropanol residue to the propionic acid group in ring D. Cyanocobalamin is formulated as a diester of phosphoric acid, the free acid group of the phosphate being neutralized by a positive charge on the cobalt atom. The coenzyme B<sub>12</sub> differs from the vitamin mainly in the absence of cyanide and the presence of an adenine nucleoside. (From Barker et al.<sup>24</sup> courtesy of the authors and the Journal of Biological Chemistry.)

### Vitamin B<sub>12</sub>

Vitamin B<sub>12</sub> is an unusual porphyrin<sup>24</sup>; two of its four pyrrole rings are linked directly rather than being joined by methene bridges, as are the other rings in the molecule and in other porphyrins. Figure 4-1 shows the structure of cyanocobalamin and indicates how the porphyrin structure is linked to the ribonucleoside of 5,6-dimethylbenzimidazole by a phosphate ester bond and by coordination of one of the nitrogens of the imidazole ring to the cobalt. Cobalt is in the position occupied by iron in the heme molecule and cyanide fills the unsatisfied valence of the

cobalt. The discovery of cobalt as a component of vitamin B<sub>12</sub> represented the first time that this mineral had been found in a pure substance of biologic origin. Vitamin B<sub>12</sub> was first isolated in 1948, but laboratory synthesis of this complex substance was not accomplished until 1973, after 11 years of work by R. B. Woodward and a team of 99 coworkers.<sup>121</sup>

Cyanocobalamin is largely an artifact of isolation procedures, the cyanide coming probably from the charcoal used. It was the first active cobalamin isolated because it is the most stable of a series of compounds. Other cobalamins differ from cyanocobalamin in the

nature of the ligand attached to the cobalt atom in the place of CN<sup>-</sup>, eg, OH<sup>-</sup> (hydroxocobalamin, vitamin B<sub>12a</sub>),<sup>72</sup> H<sub>2</sub>O (aquocobalamin, vitamin B<sub>12b</sub>), ONO<sup>-</sup> (nitrocobalamin, vitamin B<sub>12c</sub>).

Although all of the above cobalamins exhibit the nutritional properties of vitamin B<sub>12</sub>, they represent neither the naturally occurring nor the biochemically active (coenzyme) forms of the vitamin. Two vitamin B<sub>12</sub> coenzymes have been identified, namely, methyl cobalamin and 5'-deoxyadenosyl cobalamin. Both are unstable, are decomposed on exposure to light, and are easily converted to one of the other, more stable cobalamins. In liver, 70% of the vitamin B<sub>12</sub> is in the 5'-deoxyadenosyl form, and part, perhaps all, of the remainder is in the methyl form.<sup>163</sup>

**SOURCES AND REQUIREMENTS.** Vitamin B<sub>12</sub>, unlike other B vitamins, is not synthesized by higher plants but is produced by many bacteria and certain molds. As a consequence, it is found in soil and water, and in the intestines or rumina of some animals. From these sources it reaches animal tissues such as liver, glandular tissues, muscle, eggs, and, in lower concentration, cheese and milk.<sup>108</sup>

Since animal protein foods tend to be relatively expensive, the amount of vitamin B<sub>12</sub> in the diet is related to cost. The average "high-cost" diet contains 20 µg per day of vitamin B<sub>12</sub>, whereas a "poor diet" (1000 calories and 32 g of protein a day) contains about 3 µg.<sup>50</sup> Consumption of a diet with still lower amounts of vitamin B<sub>12</sub> requires avoidance not only of meat, but also of eggs and milk. In Europe and the United States, unusually strict vegetarians (vegans) are the only group known to consume such a diet.

As little as 0.1 µg of vitamin B<sub>12</sub> per day will produce a minimal hematopoietic response when given parenterally to patients with uncomplicated pernicious anemia in relapse.<sup>166</sup> This amount is so small that only such poisons as plutonium and botulinus toxin are known to be physiologically active in any smaller quantities. It has been estimated that the minimal daily adult requirement for the vitamin is 0.6 to 1.2 µg/day.<sup>93</sup>

This amount will prevent all signs and symptoms of deficiency and will maintain serum vitamin levels within normal limits. However, a somewhat greater intake (3 to 10 µg/day) might be necessary to maintain stores of the vitamin within normal limits.

Vitamin B<sub>12</sub> is an exceptionally well-stored vitamin. The total body content in normal persons has been estimated at 5000 µg (approximate range: 3500 to 11,000 µg).<sup>81</sup> The liver is the principal storage depot; it contains about 1700 µg (750 to 3000 µg), smaller amounts being distributed in other tissues. Loss of vitamin B<sub>12</sub> from the body occurs at a rate of 0.05 to 0.2% per day.<sup>21,81</sup> Thus, in a normal subject, 2.5 to 10 µg are lost per day and must be replaced if stores are to be maintained.

**ABSORPTION.** Normal absorption of vitamin B<sub>12</sub> requires that it be bound to a protein in gastric juice called "intrinsic factor." When so bound, about 70% of dietary vitamin B<sub>12</sub> is absorbed. When unbound, less than 2% is absorbed. *Intrinsic factor*<sup>73,90</sup> (IF) is an alkaline-stable, thermolabile glycoprotein; it is sensitive to peptic digestion and to storage at acid pH, especially when not bound to vitamin B<sub>12</sub> prior to digestion.<sup>20</sup> It binds B<sub>12</sub> and its analogues with a high affinity. The molecular weight of IF as a monomer is 50,000 to 60,000. In the presence of vitamin B<sub>12</sub>, two molecules of the monomer combine rapidly to form a dimer that binds two molecules of the vitamin, the complex having a molecular weight of about 115,000.<sup>92</sup>

In the hog, IF is formed in the pyloric and Brunner glands of the duodenum, but in man it is thought to be derived from the parietal cells of the fundus and body of the stomach.<sup>64,73,98</sup> Its secretion in normal persons is augmented by histamine, betazole hydrochloride and methacholine,<sup>100</sup> as well as by gastrin.<sup>171</sup> Intrinsic factor appears to be of greatest importance in omnivores. Carnivores, such as dogs,<sup>137</sup> lack the protein.

Although most nutrients are absorbed mainly in the upper segments of the small intestine, vitamin B<sub>12</sub> is absorbed principally from the lowest level of the ileum.<sup>138,151</sup> The

absorptive cells of the ileum are highly specific for this function; if they are transposed surgically to the region of the upper jejunum, they retain their capacity to absorb vitamin B<sub>12</sub>.<sup>58</sup> Upon reaching the ileum, the intrinsic factor-B<sub>12</sub> complex becomes attached to specific receptor sites on the brush border of the mucosal cells,<sup>56</sup> a process requiring a pH greater than 5.7 and the presence of divalent cations, especially calcium.<sup>60</sup> Little is known about the steps occurring between the time of attachment at the ileal receptor sites and the appearance of vitamin B<sub>12</sub> in the plasma. It is probable that the vitamin enters the absorptive cell and that the intrinsic factor remains outside.<sup>52,95</sup> Following absorption, the vitamin is associated for several hours with the mitochondrial fraction of the mucosal cells,<sup>142</sup> but the significance of this localization is not known.

Optimal absorption of vitamin B<sub>12</sub> requires pancreatic secretions. In a proportion of human subjects with pancreatic insufficiency,<sup>168</sup> as well as in partially pancreatectomized rats,<sup>169</sup> absorption of vitamin B<sub>12</sub> was found to be reduced. In both, the defect could be corrected by administration of pancreatic extracts. The mechanism of action of pancre-

atic secretions has not been established; it has been suggested that they either alter the vitamin B<sub>12</sub>-intrinsic factor complex to an absorbable form or destroy an inhibitor of absorption, such as a non-IF-protein vitamin B<sub>12</sub> binder.<sup>169</sup>

**TRANSPORT.** Most of the vitamin B<sub>12</sub> found in the circulation is bound to one of two proteins. These are designated transcobalamin I (TC I) and transcobalamin II (TC II).<sup>80</sup> Various chemical and physiologic characteristics of these two proteins are given in Table 4-I. Evidence for a third serum binder has also been reported.<sup>33,42</sup> It may be of leukocyte origin and accounts for the increase in unsaturated vitamin B<sub>12</sub>-binding capacity associated with leukocytosis.<sup>42</sup> It also is found in normal persons,<sup>33</sup> but its physiologic significance remains unknown.

Transport of vitamin B<sub>12</sub> is accomplished chiefly by transcobalamin II. This conclusion may be drawn from the following observations: (1) TC II binds nearly all of the newly absorbed or injected vitamin B<sub>12</sub>; (2) turnover of TC II-bound vitamin B<sub>12</sub> is relatively rapid<sup>96</sup>; (3) transfer of vitamin B<sub>12</sub> from TC II to tissues occurs rapidly in vivo and in

Table 4-1. Comparison of Two Plasma B<sub>12</sub>-Binding Proteins

	Transcobalamin I	Transcobalamin II
Molecular weight	115 000 <sup>75</sup> 121,000 <sup>100</sup>	36 000 <sup>75 100</sup>
Chemical nature	Glycoprotein <sup>75</sup>	
Electrophoretic mobility	$\alpha_2$ <sup>80</sup>	$\alpha_2/\beta$ <sup>80</sup>
Source	(?) Leukocytes <sup>49 51</sup>	(?) Liver <sup>105</sup>
Half life of bound vitamin B <sub>12</sub>	9-10 days <sup>99</sup>	1.5 hours <sup>99</sup>
Transfer to cells	Poor <sup>43 145</sup>	Rapid <sup>43 145</sup>
Manifestations of hereditary deficiency	Decreased serum B <sub>12</sub> levels, no anemia <sup>42</sup>	Growth failure, megaloblastic anemia <sup>79</sup>
Increased in	Chronic myelocytic leukemia, polycythemia vera <sup>88</sup>	Pregnancy <sup>88</sup>
Probable function	Storage	Transport

vitro<sup>63,143,145</sup>; and (4) vitamin B<sub>12</sub> metabolism is seriously impaired when there is hereditary lack of TC II.<sup>79</sup>

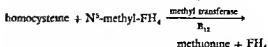
Transcobalamin I and the third serum binder appear to be related to other B<sub>12</sub>-binding proteins found in tissues and body fluids and secretions.<sup>42,75</sup> These so-called "R" proteins are immunologically similar, but apparently differ from one another in the amount of attached sialic acid.<sup>75</sup> The R protein in gastric juice accounts for the observed binding capacity of gastric juice in excess of that accounted for by intrinsic factor. Vitamin B<sub>12</sub> bound to transcobalamin I accounts for the major fraction of vitamin B<sub>12</sub> found in plasma; however, this fraction turns over relatively slowly. It has been suggested that TC I serves a storage, rather than a transport, function.

Microbiologic assay of serum using *Euglena gracilis* as the test organism has yielded widely varying values for normal adults in different laboratories, with mean concentrations ranging from 212 to 640 ng/l.<sup>24</sup> With improved methodology, a mean of 472 ng/l was found, with a range of 163 to 925. These figures are similar to those reported in *L. leichmannii* assays.<sup>101</sup> When measured by radioisotope dilution methods, values of 200 to 900 ng/l were thought to represent the normal range.<sup>113</sup> In vitamin B<sub>12</sub> deficiency due to pernicious anemia in relapse, levels of 9 to 113 ng/l were found.<sup>21</sup> Serial studies indicated that the bone marrow became megaloblastic when the serum vitamin B<sub>12</sub> concentration fell between 70 and 154 ng/l, at which time the total content of vitamin B<sub>12</sub> in the body was 100 to 660 µg.<sup>22</sup> Serum values below 100 ng/l are considered to be definitely subnormal and occur in association with a variety of megaloblastic anemias as well as with subacute combined degeneration of the spinal cord (page 612).

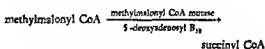
**FUNCTIONS.** A number of enzymatic reactions in microorganisms require vitamin B<sub>12</sub> coenzymes as cofactors.<sup>157,162</sup> In general, these reactions fall into two groups: those dependent on 5'-deoxyadenosyl cobalamin and those dependent on methyl cobalamin. The former usually involve intramolecular

exchanges of a hydrogen attached to one carbon atom with a group attached to an adjacent carbon atom; the latter involve transfer of methyl groups between molecules.

In mammalian tissues, only two vitamin B<sub>12</sub>-dependent reactions have been identified with certainty. The first of these is the synthesis of the amino acid methionine from homocysteine, a reaction of special interest because it requires not only methyl cobalamin but also a folate coenzyme, N<sup>5</sup>-methyl tetrahydrofolate (N<sup>5</sup>-methyl-FH<sub>4</sub>):



The second is a step in the catabolism of propionate; namely, the conversion of methylmalonyl coenzyme A (CoA) to succinyl CoA:



The relations of the coenzyme functions of vitamin B<sub>12</sub> to the metabolic consequences of B<sub>12</sub> deficiency have not been established. It is clear that there are two principal abnormalities in animals deficient in vitamin B<sub>12</sub>: defective deoxyribonucleic acid (DNA) synthesis and defective synthesis of myelin.

Two possible roles for vitamin B<sub>12</sub> in DNA synthesis have been suggested. The most likely is the so-called "methyltetrahydrofolate trap" hypothesis.<sup>127,136,177</sup> This holds that impaired DNA synthesis in vitamin B<sub>12</sub> deficiency is secondary to deranged folate metabolism. As a consequence of impaired conversion of homocysteine to methionine (see reaction above), N<sup>5</sup>-methyl tetrahydrofolate cannot be efficiently converted to tetrahydrofolate. As a result, folate becomes "trapped" in the N<sup>5</sup>-methyl form and a deficiency develops of N<sup>5</sup>N<sup>10</sup>-methylene FH<sub>4</sub>, the folate coenzyme required for thymidyllic acid synthesis (Fig. 4-3). Evidence for and against the hypothesis has been reviewed.<sup>78,136</sup> The second proposed role for vitamin B<sub>12</sub> in DNA synthesis is in the conversion of ribonucleotides to deoxyribonucleotides (the ribonucleotide reductase reaction).<sup>30</sup> In one microorganism, *L.*

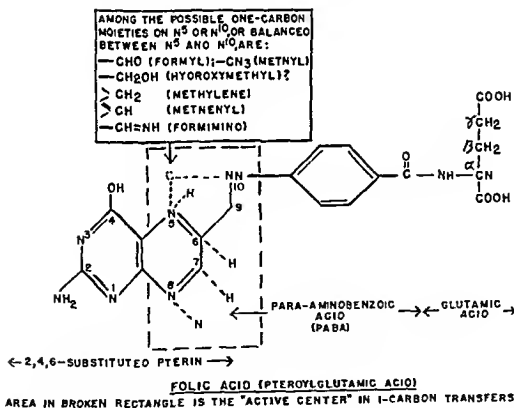
*leishmannii*, this reaction requires 5'-deoxy-adenosyl vitamin B<sub>12</sub>, whereas in another, *E. coli*, it does not. Most of the reported observations suggest that human and mammalian ribonucleotide reduction, like the *E. coli* system, is not vitamin B<sub>12</sub> dependent.<sup>157</sup>

The biochemical reactions relating vitamin B<sub>12</sub> to myelin synthesis remain uncertain. In contrast to the lesions of defective DNA synthesis, the neurologic lesions of B<sub>12</sub> deficiency cannot be relieved by folate administration; therefore, a reaction unrelated to folate metabolism is presumed to be impaired. Since the only such reaction specifically identified in man is the methylmalonyl CoA mutase reaction, efforts to attribute the neurologic abnormalities in B<sub>12</sub> deficiency to a defect in this reaction have been made.<sup>70</sup> Relevant to this hypothesis is the observation that glial

cells grown in vitamin B<sub>12</sub>-deficient tissue cultures synthesized unusual 15- and 17-carbon fatty acids.<sup>26</sup> This abnormality was ascribed to accumulation of propionyl CoA. It was suggested that the incorporation of such fatty acids into myelin might account in some way for impaired neural function.

### Folic Acid

The term "folic acid" has been used both to designate a specific chemical compound, pteroylglutamic acid, and as a general term for related compounds with similar nutritional activity. The less specific term "folate" is to be preferred for the latter meaning. The pteroylglutamic acid molecule consists of three parts: pteridine, p-aminobenzoic acid, and glutamic acid (Fig. 4-2). Most of the



BROKEN LINES OUTLINE THE BASIC 1-CARBON ACCEPTOR (5,6,7,8-TETRAHYDROFOLIC ACID) (THFA) (FH<sub>4</sub>), AND THE VARIOUS 1-CARBON-DONATING COENZYMES DERIVED FROM IT.

Fig. 4-2. The folic acid molecule. Area enclosed by broken lines is the active center in one-carbon transfers. The four N atoms are added at positions 5, 6, 7 and 8 in tetrahydrofolic acid (FH<sub>4</sub>). Various one-carbon moieties attach to the N atoms at position 5 or 10, or are suspended between both N atoms (From Herbert and Zalusky,<sup>71</sup> courtesy of the authors and Journal of Clinical Investigation)



Folate is also required for steps in de novo purine synthesis, namely, transfer of carbons 2 and 8 to the purine ring.

Of clinical interest is another reaction involving folic acid: the reaction of the degradation product of histidine metabolism, formiminoglutamic acid (FIGlu) with  $\text{FH}_4$  to form glutamate and  $\text{N}^5$ -formimino- $\text{FH}_4$  (Fig. 4-3). In severe folic acid deficiency this reaction cannot occur and FIGlu, together with its immediate precursor, urocanic acid, appears in the urine unchanged. In deficient subjects, excretion can be greatly increased by the administration of histidine prior to the collection of urine. This has been used as a test for folic acid deficiency, as has the excretion of aminoimidazol-carboxamide (AIC) (Chapter 14). AIC, in the form of ribonucleotide, is formylated in vivo to yield formaminoimidazole carboxamide, an intermediate in de novo purine biosynthesis. In folate deficiency this conversion does not occur because the formyl group is contributed by  $\text{N}^{10}$ -formyl tetrahydrofolate.

#### Vitamin $\text{B}_6$ <sup>114</sup>

Vitamin  $\text{B}_6$  is a class name for several naturally occurring derivatives of 2-methyl-3-hydroxy-5-hydroxymethyl pyridine. The

major forms of the vitamin are pyridoxine, pyridoxal, pyridoxamine, and the phosphorylated derivatives of these three compounds (Fig. 4-4).

Vitamin  $\text{B}_6$  is widely distributed in foods of both plant and animal origin. Meat and grains are excellent sources, but relatively low concentrations are found in milk. The minimum daily requirement is approximately 1.5 mg<sup>27</sup> and the Food and Nutrition Board of the U.S. National Research Council recommends an intake of 1.5 to 2 mg per day in adults and 0.4 mg per day in infants.<sup>66</sup>

In a variety of animal species, experimentally induced deficiency of vitamin  $\text{B}_6$  leads to hypochromic, microcytic anemia, iron overload, and neurologic abnormalities.<sup>67,71,109,128,147</sup> The syndrome has been described in greatest detail in swine.<sup>44,183</sup> If the diet is complete in other respects, administration of pyridoxine brings about complete relief of the anemia.<sup>46</sup> Naturally occurring dietary deficiency in man is rare, apparently because of the ubiquitous distribution of the vitamin. With an artificial, pyridoxine-deficient diet, deficiency was induced in two hydrocephalic infants, one of whom developed hypochromic, microcytic anemia.<sup>160</sup> In the 1950's, an "epidemic" of seizures in infants was ascribed to deficiency of vitamin

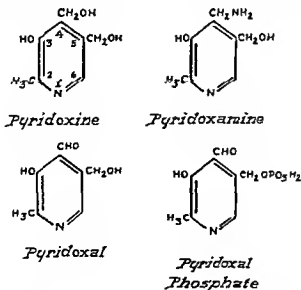


Fig. 4-4. Chemical structure of various forms of vitamin  $\text{B}_6$ .

B<sub>6</sub> in a commercial infant-feeding formula.<sup>135</sup> Experimental deficiency has been induced in adults with the antivitamin, denxypyridoxine.<sup>131</sup> Evidence of naturally occurring, mild deficiency has been observed in malabsorptive states.<sup>111</sup> Also, there is biochemical evidence for mild vitamin B<sub>6</sub> deficiency in women taking contraceptive pills<sup>152</sup> and in those who are pregnant.<sup>37</sup> In pregnancy, there is probably no consequent increase in morbidity and mortality of either mother or fetus<sup>94</sup>; in particular, the claim that hyperemesis gravidarum responds to pyridoxine therapy was not supported by a controlled evaluation.<sup>92</sup> In a number of other clinical settings (eg, malignancy, hyperthyroidism, rheumatoid arthritis, epilepsy, and others),<sup>114</sup> vitamin B<sub>6</sub> deficiency was suggested by abnormalities in tryptophan metabolism. However, a more likely explanation for these abnormalities is an induced alteration in the activity of the hepatic enzyme, tryptophan pyrrolase.<sup>23</sup>

Certain drugs, including isoniazid (INH)<sup>33,124,144</sup> and penicillamine,<sup>104</sup> inactivate or inhibit vitamin B<sub>6</sub> and may lead to symptoms of deficiency. Also, several genetic syndromes that respond to pharmacologic doses of pyridoxine, even though there is no deficiency of the vitamin, have been described.<sup>114,130,155</sup> These syndromes include pyridoxine-responsive sideroblastic anemia (page 680), "pyridoxine-dependency" seizures in infants, cystathioninuria, homocystinuria, and xanthurenic aciduria.

Vitamin B<sub>6</sub> is absorbed rapidly and completely from the normal intestinal tract. Its active coenzyme forms are pyridoxal-5-phosphate (Fig. 4-4) and pyridoxamine-5-phosphate. These may be formed *in vivo* by two enzymatic reactions: the pyridoxal phosphokinase reaction, which accomplishes phosphorylation of all three members of the B<sub>6</sub> group,<sup>123</sup> and the pyridoxine phosphate oxidase reaction, which converts either pyridoxine phosphate or pyridoxamine phosphate to pyridoxal phosphate.<sup>172</sup> These reactions have been demonstrated to occur in erythrocytes.<sup>25</sup> The principal excretory product of vitamin B<sub>6</sub> is 4-pyridoxic acid, which is

formed from pyridoxal by a nonspecific aldehyde oxidase in the liver.

The vitamin B<sub>6</sub> coenzymes participate in a wide variety of reactions almost all of which are involved with amino acid metabolism. The reactions fall into three main categories: (1) decarboxylation, (2) transamination, and (3) molecular interchanges at the bond between the amino acid  $\alpha$ - and  $\beta$ -carbons. The requirement for vitamin B<sub>6</sub> in erythropoiesis appears to be chiefly related to this vitamin's role as a cofactor in the formation of aminolevulinic acid, the rate-limiting step in heme biosynthesis (page 169).

### Riboflavin

Deficiency of riboflavin (vitamin B<sub>2</sub>) leads to anemia in a variety of species, including rats,<sup>110,129</sup> baboons,<sup>69</sup> monkeys,<sup>173</sup> and pigs<sup>184</sup> (Table 4-2). In man, anemia has been induced by riboflavin deprivation and the administration of the riboflavin antagonist, galactoflavin.<sup>112</sup>

The anemia in these experimental situations is normocytic and normochromic and is associated with erythroid hypoplasia of the bone marrow and a reduced reticulocyte count. Vacuolization of the normoblasts is observed when deficiency is severe.<sup>69,112</sup> Plasma iron transport and erythrocyte iron incorporation are reduced. Thus, the hematologic picture includes the expected morphologic and kinetic features of decreased red cell production due to erythroid hypoplasia. Leukocytes and platelets usually are unaffected. When riboflavin is administered, reticulocytosis and return of the blood hemoglobin concentration to normal ensue.

Naturally occurring riboflavin deficiency in man is almost always associated with deficiencies of other nutrients. In such situations the clinical findings usually ascribed to lack of riboflavin are angular stomatitis and seborrheic dermatitis.<sup>101</sup> Nevertheless, anemia and erythroid hypoplasia, responding to riboflavin and not to other nutrients, have been reported occasionally.<sup>68</sup>

The precise role of riboflavin in red cell production has not been defined. However,

**Table 4-2. Hematologic Characteristics of Experimental Nutritional Deficiencies in Swine**

Deficiency	Anemia		Leukopenia	Plasma Iron	Serum Copper	E P	Bone Marrow Morphology
	Type	Severity					
Protein	N	+	None	Normal	Low	Normal	Normoblastic
Lysine	N	+	None	Normal	Normal	—	Normoblastic
Tryptophane	N	++	Present	Normal	—	Normal	Normoblastic
Iron	MH	++++	None	Low	Normal	Normal	Normoblastic
Copper	MH	++++	Present	Low	Low	Normal	Normoblastic
Pyridoxine	Mi	++++	None	High	Normal	Low	Normoblastic
Niacin plus protein	N	++	None	Normal	Low	Normal	Normoblastic
Riboflavin	N	+	None	—	—	—	Normoblastic
Pantothenic acid	N	++	None	—	—	—	Normoblastic
Folate	Ma	++++	Present	High	Normal	Low	Macronormoblastic
B <sub>12</sub>	N	+	None	—	—	—	Normoblastic
Folate plus B <sub>12</sub>	Ma	++++	Present	—	—	—	Macronormoblastic with a few megaloblasts
Vitamin E <sup>132</sup>	N	++++	None	Normal	—	—	Abnormal*

Types of anemia: N indicates normocytic; MH microcytic hypochromic; Mi microcytic and Ma macrocytic.

E P refers to free erythrocyte protoporphyrin.

\*Hyperplasia with multinucleated cells.

the flavin coenzymes, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), are involved in many aspects of intermediary metabolism,<sup>150</sup> especially in oxidation-reduction reactions. Among the important enzymes requiring flavin cofactors are xanthine oxidase, succinate dehydrogenase, NADPH cytochrome c reductase, and glycolic acid oxidase. Two flavin enzymes of special importance to the red cell are glutathione reductase (page 104) and NADH methemoglobin reductase (page 105), enzymes that are required for optimal survival and function of the erythrocyte. However, any relation to erythropoiesis has not been established.

### Pantothenic Acid

In spite of its importance in the biosynthesis of hemoglobin (page 171), deficiency of pantothenic acid, experimentally induced in swine, was associated with the development of only a moderate normocytic anemia.<sup>182</sup> In these swine, severe sensory neuron degeneration was induced as well as extensive colitis. In rats, lymphocytopenia was reported.<sup>35</sup> In man, experimentally induced pantothenic

acid deficiency was found to be associated with fatigue, headache, weakness, emotional lability, impaired motor coordination, paresthesias, muscle cramps, gastrointestinal disturbances, and eosinopenia, but anemia did not develop.<sup>80</sup>

### Nicotinic Acid (Niacin)

The significance of nicotinic acid in hemopoiesis has been obscure even though a role for this vitamin has been suspected since the importance of nicotinic acid in pellagra and in acute black-tongue in dogs was first demonstrated. In the latter condition the hemoglobin may not be reduced but even may be increased when the manifestations of the disorder are at their peak. It was shown, however, that the acute symptoms could be alleviated by the parenteral administration of normal saline solution. When the saline solution was given a profound anemia made its appearance.<sup>81</sup> The anemia was found to be macrocytic, hypochromic and normocytic, normochromic in type when two different nicotinic acid-deficient diets were fed. The anemia, which was severe, was not associated with evidence of exaggerated blood destruc-

tion. Following the administration of nicotinic acid there was an immediate reticulocyte response followed by restoration of normal production of red cells. In swine maintained on a low-protein, niacin-deficient diet, normocytic anemia that responded to either protein or niacin therapy developed.<sup>39</sup> Since nicotinic acid is concerned in the synthesis of pyridine nucleotides (NAD, NADP) and thus in cell respiration, a lack of this vitamin may interfere with the respiration of immature red cells.

### Other B Vitamins

Although, by their participation in various metabolic processes, other B vitamins, such as biotin<sup>110,153</sup> and thiamin,<sup>118,186</sup> must play some role in hematopoiesis, no well-defined alterations in the blood picture have been observed in association with deficiencies of these nutrients.

### Ascorbic Acid

Ascorbic acid (vitamin C) deficiency does not occur in all species. It occurs in man, as in the monkey<sup>29</sup> and the guinea pig, because he does not possess the enzyme system required for the production of L-ascorbic acid from glucose.<sup>39</sup> Scurvy, the clinical syndrome attributed to vitamin C deficiency, frequently is associated with deficiencies of other essential nutrients. It is not surprising, therefore, that the anemia usually present in scurvy has been reported as being normocytic, macrocytic, or hypochromic, microcytic.<sup>53,74</sup> The bone marrow is usually quite cellular, with a relative increase of normoblasts,<sup>170</sup> but occasional megaloblasts have been reported.<sup>36</sup> Leukopenia is found sometimes; thrombocytopenia, rarely. Persistent reticulocytosis, indirect bilirubinemia, increased urinary urobilinogen, decreased plasma haptoglobin, and methemalbuminemia have been observed in some subjects.<sup>53,74,170</sup> It has been claimed that these changes are not solely attributable to absorption of pigment from extravascular hemolysis in the hematomas; the survival time of labeled erythrocytes from a normal com-

patible donor transfused to patients with scurvy was found to be shortened,<sup>74,126</sup> thus suggesting the presence of a hemolytic process. However, this is not found in all cases.<sup>43</sup>

Deliberately induced ascorbic acid deficiency lasting up to 39 months has not been associated with the development of anemia, even when blood loss by venesection totaled 6000 ml.<sup>54</sup> In another well-controlled study of human vitamin C deficiency, hemoglobin regeneration took place spontaneously or in response to iron therapy without the addition of ascorbic acid.<sup>117</sup>

In monkeys fed milk diets deficient in ascorbic acid, megaloblastic anemia was produced.<sup>122</sup> The experimental megaloblastosis could be eliminated or prevented by giving folic acid or folic acid without the addition of ascorbic acid and could be cured by giving ascorbic acid alone. Vitamin B<sub>12</sub> had no influence on the deficiency. If supplementary folic acid was given, when scurvy appeared the anemia was normoblastic rather than megaloblastic. As scurvy progressed, hemorrhage occurred and iron deficiency ensued. These experiments were interpreted as indicating that ascorbic acid serves no specific function in hematopoiesis, but that the requirements for folic acid and for iron are increased in scurvy. More probably, ascorbic acid exerts its effect by protecting folate in the diet from damage during heat and storage, rather than by affecting folate metabolism. In scurvy in man, low serum folic acid activity is not unusual<sup>18,53</sup> and associated folic acid deficiency no doubt often is present.

There is evidence that ascorbic acid may be required for normal iron metabolism.<sup>33,115</sup> In the Bantu with severe iron overload, the plasma iron level usually is markedly increased, but if the condition is complicated by scurvy, the plasma iron is low.<sup>33</sup> Administration of ascorbate to such patients leads to a prompt rise in plasma iron. In scorbutic guinea pigs, evidence of diminished release of iron from reticuloendothelial cells was presented.<sup>115</sup> As a result of the defect, storage iron increased in the spleen. Still other abnormalities were found in iron storage; thus, in contrast to the spleen, hepatic iron was

reduced, and in both organs the ferritin:hemosiderin ratio was decreased. The abnormalities could be corrected by administering ascorbate.

### Vitamin E and Other Fat-Soluble Vitamins

There is no evidence for any specific need for vitamin A, D, or K in erythropoiesis; however, a growing body of information indicates a requirement for vitamin E. Vitamin E was recognized as an essential nutrient as a result of the studies of Evans and Bishop.<sup>60</sup> In 1936,  $\alpha$ -tocopherol was isolated from wheat-germ oil and shown to have the biologic properties of vitamin E.<sup>61</sup> Structurally,  $\alpha$ -tocopherol consists of a heterocyclic chroman ring with a 16-carbon, aliphatic side chain (Fig. 4-5). Seven other naturally occurring forms of vitamin E have been discovered, all with considerably less activity than  $\alpha$ -tocopherol.<sup>175</sup>

Experimentally induced vitamin E deficiency in several animal species resulted in muscular dystrophy, reproductive failure, myocardial degeneration, renal tubular failure, and hepatic necrosis.<sup>134-175</sup> Hematologic manifestations were of two different types: a hemolytic anemia of varying severity and a profound disturbance of erythropoiesis. The latter was described most completely in monkeys,<sup>65,82,142</sup> but also was observed in swine.<sup>133</sup>

In monkeys, a long period of vitamin E deprivation (up to two years) was required before anemia occurred. The erythrocytes usually were found to be normocytic and normochromic, but sometimes were slightly

macrocytic. Erythrocyte survival was mildly to moderately reduced, but reticulocyte numbers were normal or only slightly increased. Erythrocytes were abnormally sensitive to hemolysis *in vitro* when exposed to hydrogen peroxide or dialuric acid. The serum iron concentration was normal. The bone marrow was hyperplastic, and the erythrocyte precursors were morphologically abnormal in that many were bi- or multinucleated. These changes were similar to those seen in congenital dyserythropoietic anemias (Chapter 19). In addition, the nuclear chromatin of erythroblasts appeared more homogeneous and more deeply stained than normal; however, the cells did not resemble megaloblasts.

After the administration of a single dose of 100 mg  $\alpha$ -tocopherol, reticulocytosis ensued and the blood hemoglobin concentration returned to normal. One form of coenzyme Q, hexahydrocoenzyme Q<sub>10</sub>, also could induce hematologic remission in vitamin E-deficient monkeys<sup>62</sup>; however, coenzyme Q<sub>10</sub>, the naturally occurring form of this coenzyme, induced reticulocytosis but little or no improvement in the anemia.<sup>65</sup>

Usually, only the hemolytic aspects of experimentally induced vitamin E deficiency have been detected in rats and mice.<sup>65</sup> Anemia was not found under ordinary circumstances, but the erythrocytes were sensitive to peroxide hemolysis *in vitro*. Furthermore, when the animals were exposed to hyperbaric oxygen, a severe hemolytic episode ensued. Administration of parenteral iron also induced hemolysis in these animals.<sup>139</sup>

Vitamin E deficiency is rare in man. The vitamin is distributed in a wide variety of foods, especially foods rich in fats and oils,

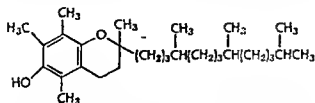


Fig 4-5. Chemical structure of  $\alpha$ -tocopherol, the principal form of vitamin E (From Silber and Goldstein,<sup>136</sup> courtesy of the authors and Grune & Stratton)

but also in grains.<sup>156</sup> The adult requirement, which may be as little as 5 mg per day or as much as 30 mg per day, probably is obtained from the great majority of diets. The need for vitamin E is related to the unsaturated fat content of the diet, and the fact that diets low in vitamin E tend also to be low in unsaturated fat is another reason for the rarity of nutritional vitamin E deficiency in man.<sup>116</sup>

In *premature infants*, vitamin E deficiency was found to be associated with low serum vitamin E levels, hemolytic anemia, and sensitivity of the erythrocyte to peroxide hemolysis, all of which could be corrected by administration of  $\alpha$ -tocopherol.<sup>77,139,149</sup> The hemolysis appeared to be aggravated by administration of iron and by the high oxygen atmospheres to which premature infants are frequently exposed.<sup>77</sup> Thus, the syndrome resembled that seen in small rodents, but had none of the dyserythropoietic features observed in monkeys and swine.

Vitamin E deficiency also has been suspected in occasional patients with *malabsorption*. They have been found to develop low serum tocopherol levels, reduced red cell survival, and increased sensitivity of erythrocyte to peroxide hemolysis.<sup>32</sup> The importance of vitamin E deficiency in the pathogenesis of the anemia of *kwashiorkor* (page 136) is disputed. Some observers have reported dramatic hematologic responses to vitamin E in patients with this disorder<sup>119</sup>; however, the detection of marrow megaloblastosis and reduced serum folate levels in these patients raises the possibility that they responded instead to folate in the hospital diet.<sup>156</sup> Furthermore, other investigators have found no response to vitamin E in *kwashiorkor* subjects.<sup>28</sup>

The role of vitamin E in biologic processes in general, or in hematopoiesis in particular, remains controversial. The best established biochemical property of this vitamin is that of an antioxidant. It has therefore been suggested<sup>167,185</sup> that, by combining with free radicals, vitamin E serves to prevent the chain of reactions called "lipid peroxidation" or rancidification.<sup>57,125</sup> By this mechanism,

oxidative damage to lipid membranes, including the red cell membrane and the membranes of mitochondria and other organelles, might be prevented. This hypothesis provides a good explanation for the hemolytic aspects of the anemia in vitamin E deficiency and for the observed sensitivity of the erythrocytes to peroxide, hyperbaric oxygen, dialuric acid, iron, and other substances capable of inducing or catalyzing lipid peroxidation. Such abnormalities might well arise from oxidative damage to the red cell membrane. The erythropoietic defects are less well explained by oxidative damage, unless they can somehow be related to impaired function of membranous organelles.

An alternative hypothesis holds that vitamin E has another, more specific metabolic function.<sup>76,154</sup> Advocates of this viewpoint maintain that efforts to establish the occurrence of lipid peroxidation *in vivo* have been generally unsuccessful. Furthermore, the antioxidant activity can be dissociated from the vitamin properties in certain compounds; eg,  $\delta$ -tocopherol is a better antioxidant *in vitro* than  $\alpha$ -tocopherol, but has much less biologic vitamin E activity.<sup>154</sup> The nature of this hypothetical function for vitamin E remains obscure. There is evidence that the vitamin may be a structural component of biologic membranes,<sup>118</sup> that it is essential to mitochondrial oxygen utilization,<sup>154</sup> and that it affects induction and repression of certain enzyme systems.<sup>134</sup> Pertinent to the role in erythropoiesis are observations suggesting a requirement for vitamin E in heme biosynthesis.<sup>41,132,134</sup> However, the hematologic findings in *anemic, vitamin E-deficient animals* are not of the kind usually found in association with disorders of heme synthesis.<sup>65</sup>

### Minerals

As a constituent of hemoglobin, iron is essential for erythropoiesis. Iron metabolism will be discussed later in this chapter (page 154). Roles in erythropoiesis have been attributed to many other minerals, but of these only copper and cobalt deserve serious consideration. It is noteworthy that commercial

iron preparations contain, as contaminants, small amounts of copper, manganese,<sup>250</sup> and even cobalt.<sup>255</sup> Anemia has been observed in offspring of magnesium-deprived, pregnant rats, but not in magnesium-deficient, adult animals.<sup>210</sup> There is no adequate evidence that manganese,<sup>212</sup> germanium, molybdenum, nickel, vanadium, or zinc has erythropoietic activity.<sup>248</sup> Calcium and phosphorus influence erythropoiesis only insofar as they affect iron assimilation (page 156).

## Copper

Anemia has been observed in experimental copper-deprivation in rats,<sup>218,250</sup> chickens,<sup>227</sup> and dogs<sup>203,231</sup>; the most extensive studies have been made in swine.<sup>231,232</sup> The anemia is characterized morphologically by hypochromia and microcytosis of the erythrocytes, changes that imply defective biosynthesis of hemoglobin. This defect appears to be a consequence of several abnormalities in iron metabolism, including impaired iron absorption, defective transfer of iron from reticuloendothelial cells and hepatocytes to plasma, and failure of the normoblast to utilize intracellular iron for hemoglobin synthesis.<sup>232</sup>

In addition to anemia, signs of copper deficiency include abnormalities in the synthesis of elastin, leading to dissecting aneurysms and intramural hemorrhages affecting major blood vessels.<sup>213</sup> Abnormalities of color and character of hair and wool have been observed as well as deformities of bones.<sup>256</sup> In second-generation copper deficiency in sheep, an extensive demyelinating neurologic disease known as "swayback" or enzootic neonatal ataxia occurs.<sup>256</sup>

## Copper Metabolism

Copper metabolism in man has been the subject of several reviews.<sup>201,209,217,216</sup> The total body copper content is about 80 mg. Copper is found in all tissues, and is probably a functional constituent of all living cells.<sup>235</sup> The highest concentrations are found in the liver, brain, and heart.

Copper is absorbed from the small in-

testine and upon entering the circulation becomes bound to albumin. This so-called "direct-reacting" fraction of plasma copper, though small in relation to ceruloplasmin copper, occupies a pivotal position in copper metabolism (Fig. 4-6). From this pool, copper is distributed to the liver, bone marrow, and other tissues. The plasma pool also receives copper from the tissues and from it is derived the copper that is excreted through the urinary tract (4%) and the intestinal wall (16%). The largest proportion (80%) of body copper loss occurs through the bile. Thus, the direct-reacting fraction reflects the turnover of copper between the gastrointestinal tract, the tissues, and the excretory routes.

The principal copper protein in plasma is ceruloplasmin, a blue glycoprotein with the electrophoretic mobility of an  $\alpha_2$ -globulin.<sup>205,225</sup> It has a molecular weight of 160,000 and seven molecules of copper (0.32%) are incorporated within its structure. Ceruloplasmin copper must be liberated from the protein before it can be measured and is therefore termed "indirect-reacting." Ceruloplasmin possesses oxidase activity toward a variety of substrates,<sup>238,259</sup> one of which is ferrous iron.<sup>239</sup> The ferrous-oxidizing (ferroxidase) activity of ceruloplasmin appears to be essential to normal iron mobilization, as will be discussed below.

The total serum copper (mean and 95% limits) is 114 (81 to 147)  $\mu\text{g/dl}$  or 13 to 23  $\mu\text{mol/l}$ . Slightly higher values are found in women (120  $\mu\text{g/dl}$ ) than in men (109  $\mu\text{g/dl}$ ). Of the total, 7 (0 to 20)  $\mu\text{g/dl}$  are "direct-reacting" (albumin-bound), and the remainder is "indirect-reacting." The normal ceruloplasmin is 31 (25 to 37)  $\text{mg/dl}$  in men, and 36 (25 to 47)  $\text{mg/dl}$  in women.

Variations in serum copper occur in different clinical conditions.<sup>230</sup> Hypercupremia is found frequently. It occurs in normal pregnancy, especially in the last trimester, in various subacute and chronic infections, and in a variety of diseases, including Hodgkin's disease, acute leukemia, aplastic anemia, hyperthyroidism, and hemochromatosis. Less consistently, it is found in chronic leukemia, lymphosarcoma, pernicious and iron-

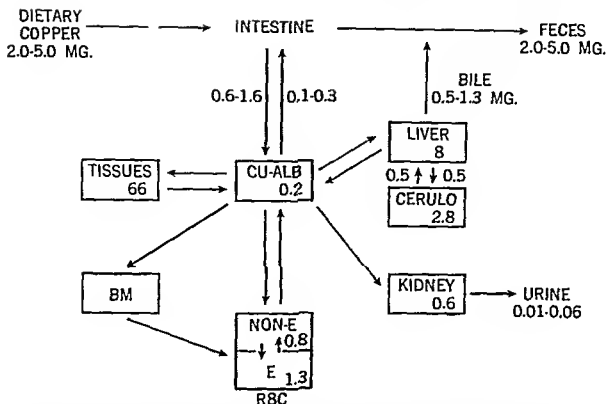


Fig 4-6. Schematic representation of some metabolic pathways of copper. The numbers in the boxes refer to milligrams of copper in each pool. The numbers next to the arrows refer to milligrams of copper traversing the pathway each day. CU-ALB direct reacting fraction, CERULO ceruloplasmin, NON-E non-erythrocyte fraction of red corpuscles (RBC), E, erythrocyte, BM, bone marrow. (Prepared by Dr. G. E. Cartwright.)

deficiency anemias (especially in infants), and in collagen disorders. These changes usually result from an increase in plasma ceruloplasmin, which, along with fibrinogen and certain other plasma proteins, is considered to be an "acute phase reactant" (see sedimentation rate, page 127).

Hypocupremia,<sup>209</sup> on the other hand, is uncommon. It tends to be found when syn-

thesis of serum proteins is low, when absorption is impaired, or when protein is being lost in large quantity (Table 4-3). It is characteristic of Wilson's disease. The level of ceruloplasmin in the serum of the newborn is about half that in the serum of adults.

Total erythrocyte copper ranges from 66 to 112  $\mu\text{g}/\text{dl}$  packed red cells. Of this, 60% is accounted for by a faintly greenish-blue protein called erythrocyte copper.<sup>253</sup> Similar or identical proteins are found in other tissues, especially the liver and brain, and the general term "cytochrome" has been proposed to apply to all such proteins.<sup>208</sup> Highly purified human erythrocyte copper has been prepared and partially characterized.<sup>256</sup> It has a molecular weight near 33,600 and contains 2 g atoms (0.38%) copper. Erythrocyte copper has been shown to have superoxide dismutase activity; i.e., it catalyzes the conversion of the superoxide anion radical ( $\text{O}_2^{\cdot-}$ ) to molecular oxygen and peroxide.<sup>256</sup> The biologic significance of this reaction is uncertain, but it may

Table 4-3. Classification of Causes of Hypocupremia

- I Dietary lack of copper
- II States associated with hypoproteinemia
  1. Kwashiorkor
  2. Celiac disease
  3. Sprue, tropical and non-tropical
  4. Idiopathic hypoproteinemia
  5. Enteropathy in infancy, in association with hyperferremia
- III Excretion from the body (nephrotic syndrome)
- IV Wilson's disease (hepato-lenticular degeneration) homozygotes and heterozygotes
- V Accelerated catabolism (?)



protect cellular elements from the damaging effects of superoxide.

Copper is known to be a constituent of a number of other enzymes, most of which are oxidases.<sup>221</sup> Examples are cytochrome oxidase, laccase,<sup>206</sup> ascorbic acid oxidase,<sup>238</sup> tyrosinase, and monoamine oxidase.<sup>263</sup>

### *Deficiency in Man*

Since copper is widely distributed in food-stuffs and is commonly present as a contaminant in water, diets of even mediocre quality contain amounts of copper that are adequate for the normal human adult.<sup>246</sup> The average diet furnishes 2 to 5 mg copper daily, and copper balance can be maintained on less than 2 mg per day.<sup>209</sup> However, cow's milk contains relatively little copper; consequently, it would seem possible for infants to become deficient under certain circumstances. Indeed, a syndrome characterized by anemia, edema, hypoproteinemia, hypoferrinemia, protein-losing enteropathy, and hypocupremia was reported in a number of infants.<sup>209</sup> However, treatment with iron alone corrected all of the observed abnormalities except the hypocupremia, whereas treatment with copper alone corrected the hypocupremia without relieving the anemia. These observations indicate that the syndrome cannot be ascribed to copper deficiency alone; indeed, iron deficiency seems to account for all of the findings except the reduced levels of copper in the serum.

In addition to the hypocupremia reported in infants, occasional cases of copper depletion to a degree sufficient to cause hypocupremia have been reported in persons with kwashiorkor, and in some with tropical and nontropical sprue, or with the nephrotic syndrome.<sup>209</sup> Furthermore, severe anemia, neutropenia, bone changes, and hypocupremia were reported in four severely malnourished infants. These infants were rehabilitated on diets high in calories but low in copper.<sup>211</sup> In two of them the response to copper was dramatic, whereas in the other two the effect of copper was less definite.

### *Role in Erythropoiesis*

The abnormalities in iron metabolism leading to the anemia of copper deficiency are only partially understood. Ceruloplasmin appears to be required for optimal flow of iron from cells to plasma. When severe hypoceruloplasminemia is induced experimentally, reticuloendothelial cells fail to release their iron at a normal rate, and this leads to hypoferrinemia in the presence of normal iron stores.<sup>232,244</sup> The abnormality can be promptly corrected by injecting ceruloplasmin intravenously.<sup>243,244</sup> Ceruloplasmin is presumed to act by catalyzing the oxidation of ferrous iron to the ferric form, which is a prerequisite for iron binding by apotransferrin.

The anemia of copper deficiency is not completely explained by defective ceruloplasmin function, however. There is evidence for two other defects. One of these affects intracellular iron metabolism in the normoblast<sup>232</sup>; as a result, iron cannot be used for hemoglobin synthesis and, instead, accumulates within the cytoplasm, forming sideroblasts. This abnormality probably relates to defective mitochondrial iron uptake.<sup>261</sup> The other erythrocyte abnormality, probably a membrane defect,<sup>233</sup> leads to shortened erythrocyte survival.<sup>207</sup> These observations imply that copper proteins are required for intracellular iron utilization and for optimal erythrocyte membrane function; however, the nature and mechanism of action of copper in these steps have not been elucidated.

### *Cobalt*

Cobalt is an essential trace element in the sense that it is a component of the vitamin B<sub>12</sub> molecule (page 137). Only microorganisms are able to utilize cobalt to form vitamin B<sub>12</sub>, and the microorganisms that inhabit the rumen of multigastric animals play a particularly important role in production of the vitamin. In geographic areas in which cobalt-deficient soils are found, such as the British Isles, Australia, New Zealand, and Florida, a malady of ruminants due to

cobalt deficiency has been observed and is variously termed "pining coast disease," "enzootic marasmus," "bush disease," and "salt sickness."<sup>235</sup> The condition is characterized by unthriftiness, progressive wasting, and profound macrocytic<sup>235</sup> or normocytic<sup>234</sup> anemia as well as hemosiderosis.<sup>255</sup> It is not accompanied by neurologic changes. The disorder can be relieved when cobalt is administered orally, but not when it is given intravenously. The significance of these findings was clarified by the discovery that cobalt is incorporated into vitamin B<sub>12</sub> in the rumen.

The experimental production of cobalt deficiency<sup>222</sup> is made difficult by the low requirement for cobalt. Cobalt deficiency does not seem to occur naturally except in ruminants. It is noteworthy that nonruminant, herbivorous animals remained healthy when confined to pastures on which cattle and sheep developed enzootic marasmus.<sup>235</sup> Interestingly, the livers of ruminants are a more potent source of vitamin B<sub>12</sub> than are those of pigs or rats.<sup>232</sup>

Cobalt is ubiquitous. It is found in small concentrations in foods, water, beverages, and in mammalian tissues and body fluids.<sup>247</sup> Concentrations in tissue are greatest in the liver, heart, and adipose tissue. The dietary intake of cobalt ranges from 140 to 580 µg/day and urinary excretion equals about 85% of the intake. During absorption, cobalt apparently follows pathways also utilized by iron<sup>215,257</sup> (see Chapter 16, page 630), and many of the factors that enhance iron absorption have the same effect on cobalt. Because of its ubiquitous distribution in food, the fact that it is readily absorbed and excreted and does not accumulate with age, and its ability to act as a catalyst and form chelates,<sup>247</sup> cobalt has been suspected as being an essential trace metal in ways other than as a constituent of vitamin B<sub>12</sub>. Nevertheless, no other requirement for nor function of cobalt has been discovered.

### *Cobalt Polycythemia*

The administration of cobalt in quantities far in excess of the normal dietary require-

ment results in the production of polycythemia in many species of animals.<sup>248</sup> This is due to a true increase in red cell mass<sup>237</sup> and is accompanied by reticulocytosis, hyperplasia of the bone marrow, and increased erythropoietic activity in the liver and spleen.<sup>218</sup> It is thought that cobalt acts by inhibiting enzymatic activities that deal with the transport of oxygen and that the resulting tissue anoxia produces polycythemia. The cobaltous ion is known to have a marked inhibitory action *in vitro* on the endogenous respiration of a number of tissues. By attaching irreversibly to the reduced disulfide groups of the cofactor, lipoic acid, cobalt blocks the conversion of pyruvate to acetyl coenzyme A and of  $\alpha$ -ketoglutarate to succinate—two steps of vital importance to cellular respiration.<sup>260</sup> The influence of cobalt in producing polycythemia can be lessened maternally by the inclusion in the diet of sufficient sulfur-containing amino acids.<sup>224</sup> These substances, especially cysteine,<sup>237</sup> are known to chelate with cobalt.

Other studies suggest that cobalt stimulates the formation of erythropoietin (page 180). This seems to be the mechanism whereby cobalt overcomes the anemia induced in animals by hypophysectomy<sup>214</sup> and possibly that of protein deficiency.<sup>237</sup> However, direct stimulation of erythropoiesis in the isolated hind limb by cobalt also has been reported.<sup>219</sup>

The administration of cobalt in large amounts simultaneously with the experimental induction of inflammation was found to prevent the development of anemia resulting from the inflammation.<sup>262</sup> Likewise, in patients with various types of anemia refractory to other forms of antianemic therapy, such as anemia associated with infection,<sup>264</sup> renal disease,<sup>223</sup> or cancer,<sup>251</sup> the daily oral administration of 20 to 300 mg, but more usually 60 to 150 mg, of cobaltous chloride is associated with reticulocyte increases and some relief of the anemia. Claims that hemoglobin regeneration in patients with iron-deficiency anemia is more rapid when cobalt-iron mixtures are given than the regeneration resulting from iron therapy alone are not convincing. Also, reports that cobalt im-

proves iron utilization in the anemia of prematurity<sup>214, 212</sup> fail to offer any observations on the effect of iron therapy as compared with iron-cobalt therapy in parallel series of infants. Cobalt therapy relieved the anemia in a patient with "acquired erythrocytic hypoplasia,"<sup>249</sup> but a significant effect in patients with hypoplastic or aplastic anemia seems to be the exception<sup>204</sup> rather than the rule.

The prolonged administration of cobaltous chloride in the doses cited may be associated with anorexia, nausea, and vomiting. More serious is the possibility of cardiac toxicity. A fulminating cardiomyopathy occurred in a number of individuals following consumption of beer to which cobalt had been added as an antifoaming agent.<sup>202</sup> Less frequent ill effects include flushing of the face and extremities, skin rash, tinnitus and nerve deafness, substernal pain, and even thyroid hyperplasia associated with thyroid hypofunction.<sup>229</sup>

Not only is cobalt potentially toxic, but also patients with anemia associated with infection or cancer who were treated with cobalt seemed to derive no real benefit even though the anemia became less severe. In patients with renal anemia,<sup>223</sup> cobalt therapy was associated with improved appetite and greater tolerance for medications necessary to correct electrolyte abnormalities. However, the increased blood values promptly declined to pretreatment levels when cobalt therapy was discontinued. It appears, therefore, that the uses of cobalt as a therapeutic agent are very limited.

## Zinc

Zinc<sup>210</sup> is found in red corpuscles, leukocytes, and plasma. The zinc in red corpuscles is mainly attributable to the content of carbonic anhydrase in these cells,<sup>258</sup> but a portion of the zinc in erythrocytes exchanges freely with the body pool of zinc.<sup>215</sup> The zinc content of erythrocytes is increased in megaloblastic anemia, in chronic leukemias, acute lymphoblastic and monocytic leukemia, and in myeloid metaplasia.<sup>220</sup> A reciprocal rela-

tionship between the levels of zinc in erythrocytes and leukocytes in various diseases has been noted. Low values in leukocytes were found in patients with conditions in which erythrocyte values were high. It is assumed that the zinc metalloprotein of leukocytes is an enzyme or group of enzymes, but no correlation with a number of specific enzymes could be established.<sup>258</sup>

Although zinc deficiency was thought to be the cause of growth retardation, hypogonadism, and partial adrenal hypofunction in clay-eating patients in Iran and in Egypt, it was not found to be related to the iron-deficiency anemia also encountered in many of these subjects.<sup>241</sup> "Meat anemia," described in mice, was found to be due to dietary lack of copper associated with an excess of zinc.<sup>225</sup>

## Iron Metabolism

### Total Body Iron

The body iron content of a normal, adult male is approximately 50 mg/kg body weight, whereas that of adult women is about 35 mg/kg.<sup>316</sup> This male-female difference reflects the high incidence of iron deficiency in women and should not be taken to mean that there are fundamental differences in iron metabolism between the sexes. Of the total body iron, only a minute portion (less than 0.1%) is found in the plasma, where it is carried in bond with a serum  $\beta_1$  globulin (transferrin). The remainder is either bound in a porphyrin ring as a part of hemoglobin, myoglobin, or one of the heme enzymes (Table 4-4), or is laid aside as storage iron. The storage forms of iron, ferritin and hemosiderin, constitute normally about 30% of the body iron, or about 1 g in men. Smaller stores, 200 to 400 mg, are found in women.<sup>316</sup>

### Iron Balance

The total iron content of the body tends to remain fixed within relatively narrow limits, otherwise siderosis or iron deficiency oc-

Table 4-4. Approximate Composition of the Iron-Containing Compounds in the Human (70-kg Man)<sup>344</sup>

Compound	Total Amount (g)	Iron Content (g)	% of Total Fe
<i>Iron porphyrin (heme) compounds</i>			
Blood hemoglobin . .	800	2.67	66.7
Muscle hemoglobin (myoglobin)	40	0.14	3.3
<i>Heme enzymes</i>			
Cytochrome c	0.8	0.0034	0.08
Catalase	5.0	0.0045	0.11
Cytochrome A, A <sub>1</sub> , B	—	—	—
Peroxidase .	—	—	—
<i>Non-heme compounds</i>			
Transferrin (siderophilin)	7.5	0.003	0.07
Ferritin . .	3.0	0.7-1.5	—
Hemosiderin	—	—	—
Total available iron stores	—	1.2-1.5	30.0
Total iron . .	—	4.0	100

curs. It follows that losses of iron must be matched by the absorption of iron from food. Iron is not "excreted" in the usual sense of the word. It is lost from the body only when cells are lost, especially epithelial cells from the gastrointestinal tract. Urinary iron amounts to less than 0.05 mg/day, and is largely accounted for by desquamated cells. In women, menstrual flow constitutes an important additional route of iron loss. On the basis of long-term studies of body iron turnover, the average daily loss of iron has been estimated at about 1.0 mg (range 0.6 to 1.6 mg) in normal, adult men and non-menstruating women.<sup>345</sup> About twice this amount is lost in menstruating women.<sup>350,362</sup> In pregnant women, the rate of iron loss is about 3½ times as great as in normal men (Table 17-6, page 648).

In the normal situation, these losses are balanced by an equivalent amount of iron absorbed from the diet. The average intake of iron in the American diet is about 6 mg per 1000 calories<sup>316</sup> or between 10 and 30 mg per day, but there is much greater variation than this in different parts of the world and under various circumstances.<sup>374</sup> Assuming that 1 mg enters the body to balance the amount lost, only 5 to 10% of dietary iron is absorbed. This proportion can increase some three- to fivefold if iron stores are

depleted. Conversely, the proportion absorbed decreases in states of iron overload.<sup>407</sup> Thus, iron balance is unique in that it is achieved by control of absorption rather than control of excretion. How this control is accomplished has been the subject of much controversy.

Iron is absorbed chiefly in portions of the intestine proximal to the mid-jejunum, and absorptive capacity is much less in more caudal intestinal segments.<sup>408</sup> This localization is partly related to such intraluminal factors as pH and redox potential. However, in experiments with isolated gut loops<sup>353</sup> and in *ex vivo*, inverted sac preparations,<sup>391</sup> as well as in studies of isolated mucosal cell brush borders,<sup>346</sup> a decreasing capacity to absorb iron as more caudal segments were studied could be demonstrated without relation to intraluminal factors.

In man and other omnivorous mammals, there appear to be at least two distinct pathways for iron absorption, one for iron attached to heme<sup>314,402,409</sup> and another for iron in the form of ferrous ion (or, possibly, soluble ferrous chelates).<sup>375</sup> Dietary iron must be converted to one of these two forms in order to be absorbed. Heme iron is derived from the hemoglobin, myoglobin, and other heme proteins in foods of animal origin. Exposure to the acid and proteases of gastric

juice frees the heme from its apoprotein, whereupon the iron is probably oxidized to the ferric state, forming hemin. This molecule enters the mucosal cell intact<sup>405</sup> and then is metabolized as will be discussed below. Other forms of food iron must be converted to the ferrous form to be absorbed.<sup>375</sup> Since the ease with which this conversion is accomplished differs according to the nature of the iron compounds, the "availability" of food iron is quite variable.<sup>374</sup> In foods derived from grains, iron often forms a stable complex with phytates and only small amounts of such iron can be converted to the absorbable form.<sup>393</sup> Similarly, the iron in egg yolk is not readily absorbed, probably because it is complexed with phosphates or phosphoproteins.<sup>374</sup> The exact nature of iron in many other foods is not known. However, it appears to be mainly in the ferric state, much of it as ferric hydroxide or loosely bound to organic molecules such as sugars, citrate, lactate, and amino acids.

Various *intraluminal factors* influence non-heme iron absorption,<sup>323</sup> but have no effect on heme iron. These factors act by affecting the ease with which the ingested forms of non-heme iron are converted to, or maintained in, the ferrous state. Thus, reducing agents, such as ascorbic acid and cysteine, facilitate absorption. The acid gastric juice is a medium in which solubilization and reduction of iron are favored; consequently, absorption of ferric iron is impaired in subjects with gastrectomy or achlorhydria,<sup>319,355,356,373,379</sup> as well as in rats with radiation-induced achylia gastrica.<sup>379</sup> However, intrinsic factor is not required for iron absorption.<sup>352</sup> Compounds forming soluble ferrous chelates, such as fructose and other keto sugars<sup>323</sup> and certain amino acids,<sup>404</sup> increase absorption. On the other hand, compounds forming insoluble complexes with iron, such as the aforementioned phytates and phosphates as well as oxalates, decrease absorption. Calcium in the diet may facilitate iron absorption by competing with iron in the formation of these complexes.<sup>303</sup>

Certain gastrointestinal secretions have been alleged to be among the intraluminal

factors influencing iron absorption. Initial reports indicated that pancreatic secretions contain a factor that decreases iron absorption.<sup>314</sup> It was proposed that the pancreas might play an important role in regulation of iron absorption and in the pathogenesis of hemochromatosis.<sup>326</sup> However, evidence for this hypothesis has been found wanting.<sup>323</sup> Similarly, a gastric iron-binding component called "gastroferrin" was found to be decreased in patients with iron deficiency and hemochromatosis,<sup>327</sup> but the physiologic importance of this substance, possibly a carbohydrate,<sup>378</sup> remains to be demonstrated.

Iron absorption takes place in two distinct steps: (1) mucosal uptake, and (2) transfer of iron from the mucosal cell to the lamina propria, where it enters plasma.<sup>358,391,407</sup> Both steps probably represent energy-dependent, active transport processes, although some have suggested that a non-energy-dependent, carrier-mediated mechanism exists.<sup>353</sup> Mucosal uptake occurs rapidly at the brush border of the mucosal cell.<sup>307,346</sup> Within the next few hours, the newly acquired iron is found to be associated with the rough endoplasmic reticulum and with free ribosomes.<sup>307</sup> Some of it may be present in cytoplasm as unbound iron salts or complexes of low molecular weight.<sup>394</sup> A variable proportion of iron taken into the cell is delivered to the plasma within a few hours.<sup>383</sup> The remainder probably is incorporated into mucosal ferritin,<sup>315,394</sup> an intracellular iron protein that will be discussed in a later section (page 162). Much of the mucosal ferritin iron appears to be sloughed into the intestine with the mucosal cell after the latter has completed its three- to four-day life span.<sup>324</sup> However, a "delayed phase" of iron absorption has been observed that occurs between 3 and 24 hours and may represent mobilization from ferritin or other intracellular binding sites.<sup>383</sup>

When heme iron enters the cell, the porphyrin ring is cleaved by an enzyme,<sup>405</sup> possibly xanthine oxidase,<sup>328</sup> and the liberated iron thereafter follows the same pathways as those utilized by ionic iron. A small proportion of the heme iron may pass into the

plasma intact<sup>405</sup> and become bound to the heme-binding protein, hemopexin.

Since the total body iron content depends so greatly on absorption of iron, the mechanisms by which the rate of absorption is regulated have been of great interest for many years. Two factors are of prime importance in determining absorptive rate. The first of these is the amount of storage iron—when it is depleted, iron absorption is increased; when it is excessive, iron absorption is decreased. The second factor is the overall rate of erythropoiesis, whether “effective” or “ineffective.” Iron absorption is increased when the red cell production rate is increased and absorption is decreased when production is decreased.

The manner in which these two factors influence the function of the mucosal cell remains the subject of active speculation and investigation. The “mucosal block theory” held that there are a limited number of iron acceptor sites within the intestinal mucosa; if these are saturated, either exogenously or endogenously, a block in further iron absorption results.<sup>348</sup> In an extension of the theory, apoferritin was postulated to be the iron acceptor and ferritin synthesis was thought to be an intermediate in the absorptive process.<sup>344</sup> This theory gradually lost favor, however, when it was demonstrated that iron is absorbed even when tissues are laden with deposited iron.<sup>331,334,397</sup>

A modified version of the mucosal block theory has been put forward on the basis of a series of experiments in rats<sup>317,324,407</sup> (Fig. 4-7). These studies showed that endogenous iron was incorporated into mucosal cells as they were formed in the crypts of Lieberkühn. It was proposed that the subsequent absorptive behavior of the mucosal cell is regulated by the amount of this “messenger iron,” more than normal being incorporated when iron stores are excessive or erythropoiesis is depressed, and reduced amounts being incorporated in iron-deficiency states or during accelerated phases of erythropoiesis. Depending on this “message,” iron taken up from the gut can either proceed into the plasma or be incorporated into ferritin to be

sloughed at the end of the life span of the mucosal cell. The failure to demonstrate significantly reduced amounts of non-heme iron in mucosal epithelial cells in iron-deficient rats with increased iron absorption has been cited as evidence against the hypothesis.<sup>306</sup> Others, however, found mucosal iron to be reduced in a supernatant fraction of mucosal homogenate from both iron-deficient and hemolyzing animals.<sup>394</sup> It is possible that the “messenger iron” enters a critical subcellular location such as the mitochondria,<sup>410</sup> or that it partially saturates a “carrier” essential to absorption.<sup>383,384,400</sup>

Efforts also have been made to demonstrate that the control of iron absorption is humoral in nature. No such humoral factor could be found in plasma from iron-deficient animals.<sup>309</sup> However, evidence of a possible inhibitor of iron absorption was found in the plasma of iron-loaded rats.<sup>368</sup>

### The Iron Cycle

The metabolism of iron is dominated by its role in hemoglobin synthesis. In this process, iron is utilized over and over again, so that, for the most part, the internal movements of iron may be described as a cycle (Fig. 4-8). Central to this cycle is the plasma iron compartment, in which iron is bound to a transport protein, transferrin. Iron moves from plasma to cells that have the capacity to make hemoglobin. When synthesis is complete, the iron, now in the form of hemoglobin in mature red corpuscles, is delivered to the circulation. At the end of their 120-day life span, the red cells are engulfed by macrophages of the reticuloendothelial system (RES) where the iron is extracted from the hemoglobin. Some of this iron may remain stored in the RES as ferritin or hemosiderin, but most is delivered to the plasma where it becomes bound to transferrin, completing the cycle. In the normal adult male, about 30 mg of iron completes the iron cycle each day. An additional small amount of iron, probably less than 2 mg, leaves the plasma each day to enter hepatic parenchymal cells and other tissues. Here, the iron is utilized for tissue

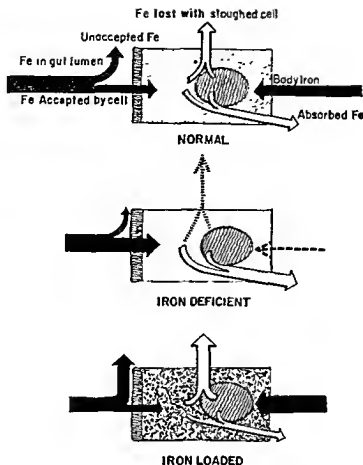


Fig 4-7 Concept of the control of iron absorption by the intestinal mucosa. The columnar epithelium of the small intestine regulates iron absorption. In the normal state, the mucosal cell contains iron supplied from body stores and this reduces the amount of iron that can be absorbed from the bowel. As a result, some iron remains in the gut lumen and is lost. Iron that enters the cell, but is not absorbed into the blood stream, also is lost when the columnar cell is sloughed at the end of its life span.

In iron deficiency, there is little to prevent entry of iron into the villous epithelial cells, nor is it retained there. It passes readily into the blood.

In iron-loaded subjects, the body iron that is incorporated into the epithelial cells is eventually lost as these cells are sloughed, but during the life span of the cells its presence inhibits the entry of iron into the cells. (From Crosby,<sup>324</sup> courtesy of the author and Grune & Stratton.)

heme proteins, such as myoglobin and the cytochromes. In the paragraphs to follow, each of the major components of the iron cycle and the factors governing the movement of iron from one to another will be discussed in greater detail.

#### Plasma Iron Transport

The plasma iron-binding protein, transferrin<sup>364</sup> ("siderophilin"), is a pink glycoprotein

with the electrophoretic mobility of a  $\beta_1$ -globulin. There is rather poor agreement as to its molecular weight, reported values ranging from 68,000 to 95,000.<sup>359</sup> With gel electrophoresis, a number of genetic transferrin variants have been identified, presumably differing from one another in amino acid sequence.<sup>392</sup> They are inherited in an autosomal, codominant fashion, and all appear to share the same important chemical and physiologic functions.

Transferrin is synthesized chiefly in the liver by the parenchymal cells,<sup>359,401</sup> but additional synthesis may occur in macrophages of lymphoid tissue and in such ectodermal glands as the submaxillary and mammary glands, as well as in the ovary and testis.<sup>401</sup> Several groups of investigators have labeled transferrin with <sup>131</sup>I in order to study its distribution and kinetics.<sup>304,322,359</sup> These studies show that the protein is about equally distributed between plasma and extravascular sites; exchange takes place between the two compartments at the rate of about 5% per hour. Transferrin turnover follows first-order

kinetics with a half-disappearance time of about 10 days. The sites and mechanism of catabolism have not been clearly delineated.

The concentration of transferrin in the plasma is about 2.5 g/l. More commonly, transferrin is quantified in terms of the amount of iron it will bind, a measure called the "total iron-binding capacity" (TIBC). In the normal subject, the plasma iron concentration is about 18  $\mu\text{mol/l}$  (100  $\mu\text{g/dl}$ ) and the TIBC is 56  $\mu\text{mol/l}$  (300  $\mu\text{g/dl}$ ). Thus, only about one third of the available transferrin binding sites are occupied. There is a diurnal variation in plasma iron concen-

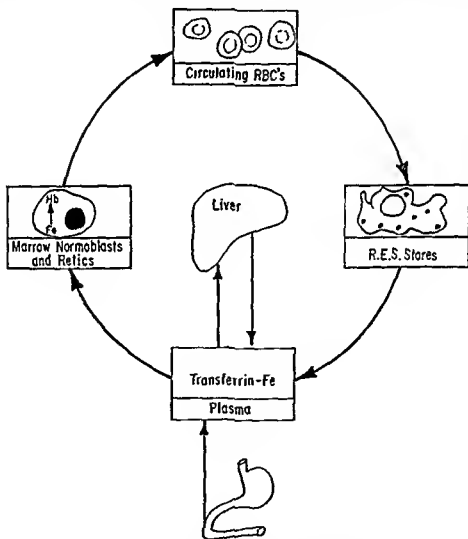


Fig 4-8. The iron cycle in man. Iron (Fe) travels from plasma to the marrow where it is incorporated into hemoglobin. It is then released with the mature red blood cells (RBC's) into the circulation. After a life span of about 120 days, the red cell is engulfed by cells of the reticuloendothelial system (RES). Here, the iron is extracted from hemoglobin and returned to plasma where it becomes bound to transferrin, completing the cycle.



tration, with highest values in the morning and the lowest in the evening. No diurnal variation occurs in TIBC. Data on normal values for plasma iron and TIBC are given in the Appendix (Table A-21), and expected variations in disease are discussed in Chapter 16.

Transferrin binds two molecules of trivalent (ferric) iron at spatially separated sites on the protein.<sup>359</sup> Most studies have indicated that the two binding sites have identical physical and chemical characteristics.<sup>359</sup> However, more recent observations with electron paramagnetic resonance, and also Mossbauer and optical difference spectroscopy, suggest that the two sites differ from one another.<sup>299, 302, 387</sup> Although the exact nature of the sites is not known, it is probable that three tyrosine and two histidine residues attach to the iron and that the tertiary configuration of the protein is essential to optimal binding.<sup>359</sup> One mole of bicarbonate is taken up and three moles of hydrogen ion are liberated for each mole of iron bound. The characteristic pink color of transferrin, with an absorption peak at a wavelength of 465 nm, is a function of the interaction between iron, bicarbonate, and the binding site. Under physiologic circumstances, the affinity of transferrin for iron is very great. The intrinsic binding constant is of the order of  $10^{36}$ , a value much higher than for other known iron chelating agents. Affinity of transferrin for iron can be decreased by lowering the pH or by reducing the iron to the divalent (ferrous) form. Other transition metals, such as copper, chromium, manganese, and cobalt, can be bound by transferrin,<sup>300</sup> but with much less affinity than iron.

The transferrin-mediated delivery of iron to red cell precursors imparts direction to the flow of iron. Unbound iron is not oriented toward a specific target<sup>333</sup>; instead, it leaves the plasma rapidly and becomes distributed to many tissues with no regard for their iron needs. The biologic importance of transferrin is illustrated by the findings in patients with congenital atransferrinemia,<sup>343, 351</sup> whose red cells had the morphologic stigmata of iron deficiency, but whose tissues were laden with iron. Thus, although iron continued to be

absorbed, it could not be provided to the developing normoblasts.

In the course of delivering iron to erythrocyte precursors, transferrin becomes transiently bound to the cells,<sup>357</sup> a phenomenon that to some degree is species specific.<sup>363</sup> Only cells that are capable of synthesizing hemoglobin bind transferrin. Thus, 75  $\mu$ g of transferrin are bound by 1.0 ml of reticulocytes (about 50,000 molecules of transferrin per cell), but only 0.6  $\mu$ g is bound by 1.0 ml of mature erythrocytes. Since the binding process could be inhibited by pre-incubation of the reticulocytes with trypsin or neuraminidase, and since bound transferrin was attached to "stroma" after hemolysis, it was proposed that there are specific receptor sites for transferrin located on the red cell membrane.<sup>357, 359</sup> It is thought that, after binding occurs, iron is released into the cell and apoferritin is liberated into the surrounding plasma. However, the possibility that both the iron and protein enter the cell has not been excluded completely.<sup>376</sup>

Fletcher and Huehns have proposed that the two transferrin iron-binding sites behave differently with respect to tissue iron transfer.<sup>339</sup> One site, designated "A," releases its iron preferentially to erythroblasts, whereas the "B" site releases its iron preferentially to other tissues, such as the liver. Although additional investigation is required before this idea can be accepted, some support for it has come from *in vitro*<sup>341</sup> and *in vivo*<sup>305</sup> studies.

The nature of the interaction between transferrin and the erythrocyte at the molecular level is not yet understood. The kinetics of the reaction(s) are similar to those of an enzymatic or a carrier-mediated reaction,<sup>362</sup> but no enzyme or carrier has been identified. There is indirect evidence that at least two distinct processes are involved: transferrin uptake, and release of iron from transferrin.<sup>376</sup> The first may represent passive attachment to (or diffusion across) the membrane since it requires little energy and is inhibited by agents like trypsin, which act primarily on the membrane.<sup>332</sup> The second appears to require an intact, mitochondrial electron-transport chain and can be inhibited

by a variety of agents that interfere with mitochondrial function. Reagents that react with sulfhydryl groups also inhibit the iron delivery process, but it is not clear whether they do so by affecting transferrin uptake<sup>376</sup> or iron release.<sup>332</sup>

The rate of entry of iron into normoblasts also is intimately related to heme biosynthesis.<sup>386</sup> The addition of free heme inhibits reticulocyte iron uptake *in vitro*. Furthermore, a decrease in intracellular free heme concentration induced by inhibitors of heme synthesis (eg, isoniazid) leads to increased iron uptake, and an increase in intracellular free heme induced by inhibitors of globin synthesis (eg, cycloheximide) has the opposite effect. These phenomena may reflect a feedback-inhibition system that regulates the supply of iron according to the needs of the cell for hemoglobin synthesis.

The transferrin system undoubtedly represents the most important pathway by which iron gains access to the red cell. However, an alternate route has been proposed on the basis of electron microscopic observations.<sup>308</sup> Bessis and Breton-Gorius described islands of normoblasts in bone marrow centered around large reticuloendothelial cells.<sup>308</sup> They consider these to be distinct anatomic units whereby normoblasts obtain iron as well as other essential building materials. The surface of the normoblast presents little invaginations that become tiny vacuoles penetrating the cytoplasm of the normoblast (Chapter 3, page 85). Small particles of cytoplasm of the central reticuloendothelial cells appear to be aspirated in this way, a process that Bessis and Breton-Gorius have termed "rhopheocytosis." Others have confirmed these findings and have presented evidence that the direction of movement is, as Bessis and Breton-Gorius proposed, from RE cell to normoblast.<sup>399</sup> The quantitative significance of this process is still to be determined.

#### Iron Metabolism within Normoblasts

The fate of iron after having entered the immature erythrocyte is known in broad outlines, but much remains to be learned about

the biochemical steps and intermediates in the process. In the normal subject, about 80 to 90% of the iron that enters the cell ultimately is taken up by mitochondria and incorporated into hemoglobin. Most of the remainder is accounted for as ferritin,<sup>369,388,411</sup> the chemical nature of which will be discussed in a later section (page 162). The presence of ferritin in erythrocytes may sometimes be detected by means of the Prussian blue reaction.<sup>329,330</sup> Normoblasts with Prussian blue-positive (siderotic) granules are called *sideroblasts*, and, if the granules persist after denucleation, the mature cells are called *siderocytes*. In the normal person, about half of the normoblasts are sideroblasts, each containing less than five small granules.<sup>330</sup> By electron microscopy (see pages 33, 87), these "normal" siderotic granules are seen to be aggregations of ferritin, often surrounded by a membrane ("siderosomes").<sup>308,329</sup> Isolated molecules of ferritin, not surrounded by a membrane, may also be seen by means of electron microscopy, but not with light microscopy.

Another kind of sideroblast, the "ringed" sideroblast, is found only under pathologic circumstances<sup>312</sup> ("sideroblastic anemias," Chapter 18). In the ringed sideroblast, the siderotic granules form a full or partial ring around the nucleus, and on electron microscopy the iron is found to be deposited in mitochondria.<sup>368</sup> The chemical form of the mitochondrial iron is not known, but it does not appear to be ferritin.

The two kinds of siderotic granules can be induced experimentally in swine.<sup>329</sup> Vigorous phlebotomy leads to the formation of siderocytes that are reticulocytes containing ferritin aggregates ("R-S cells"). Production of vitamin B<sub>6</sub> deficiency leads to siderocytes characterized by iron-laden mitochondria. The spleen is required for removal of the iron-laden mitochondria, but not of the "normal" cytoplasmic ferritin aggregates. The latter are removed by solubilization and extrusion of ferritin into the surrounding media, a process requiring active cellular oxidative metabolism.<sup>329,359</sup> Variation in numbers of sideroblasts and siderocytes in disease is discussed in Chapters 16 and 18.

In the red cell, ferritin appears to function as a component of a mechanism by which unneeded iron can be temporarily stored and later excreted. This function may be inferred from two observations: (1) once iron is incorporated into ferritin it does not appear to be available for heme synthesis,<sup>388,411</sup> and (2) as noted above, the cell is able to discharge ferritin into the surrounding plasma. Not excluded, however, is the possibility that the ferritin iron may be made available to the cellular hemoglobin-synthesizing apparatus under special pathologic or physiologic circumstances.

Intracellular iron compounds other than heme and ferritin are presumed to exist, but have not been identified. One proposed intracellular iron-transport protein<sup>347</sup> was later acknowledged to be an artifact.<sup>388</sup> There is fragmentary evidence for an iron compound of small molecular weight to which iron is attached as it passes from transferrin to hemoglobin.<sup>369,388,411</sup> Chelates of iron with ATP or ADP possess many of the properties expected of such a compound,<sup>312,369,398</sup> but iron citrate<sup>301</sup> and other soluble iron chelates also are candidates for this role. Furthermore, the inhibition of reticulocyte heme synthesis by the ferrous chelating agent, bipyridine,<sup>388</sup> suggests that iron may exist, at least transiently, in ionic form.

### Iron Metabolism in the Reticuloendothelial System

At the end of its life span, the erythrocyte is engulfed by reticuloendothelial (RE) cells. These cells acquire most, and possibly all, of their iron by this means. They probably do not accept measurable amounts of iron from transferrin,<sup>335,359</sup> although a contrary view was suggested by one study.<sup>367</sup> Within the RE cell, the erythrocyte membrane is broken, and hemoglobin iron is oxidized to the trivalent state, forming methemoglobin (page 105). In this form, the heme and globin dissociate from one another relatively easily. Then, iron is liberated from heme, a reaction that requires an enzyme, microsomal heme oxygenase. This reaction is discussed in more

detail in relation to the metabolism of bile pigment (page 208).

The RE system is the principal source of supply of iron to the plasma. In order to meet a variable demand for iron, the RE system maintains a sizable storage pool in the form of ferritin and hemosiderin. Under equilibrium conditions, ie, when the amount of iron that enters the RE cell approximates that which leaves it, there is relatively little interchange between the newly liberated iron and the storage forms.<sup>380</sup> Instead, iron from recently destroyed erythrocytes passes quickly through the RE cell and is presented to the plasma at the cell surface. The intermediate iron compounds in this process, if any, are not known. However, at the cell-plasma interface, the iron probably is in the form of ferrous ion, and it must be oxidized in order to be bound to transferrin. The ferrous oxidation (ferroxidase) reaction is catalyzed by the plasma copper protein, ceruloplasmin.<sup>239,243,244</sup> (page 150).

In non-equilibrium situations, iron either enters or leaves the stores. When the red cell mass is expanding and erythrocytes are being produced more rapidly than they are being destroyed, iron is mobilized from RE stores. The amount of iron leaving the RE cell then exceeds that which enters. When red cell destruction exceeds production, iron is deposited in stores, and the amount of iron entering the RE cell exceeds that which leaves. It is apparent that mechanisms must exist for coupling the rate at which iron leaves the RE cell to the rate of red cell production. The nature of the control mechanism remains unknown.

The two iron storage compounds are ferritin and hemosiderin. The ferritin molecule is roughly spherical in shape and consists of a protein shell surrounding a ferric hydroxyphosphate core.<sup>319</sup> The protein portion (apoferritin) has a molecular weight of about 460,000 and is probably made up of 24 polypeptide subunits, each with a molecular weight of about 18,500. When fully saturated with iron, ferritin has a molecular weight near 900,000 and contains about 5000 iron atoms per molecule (about 23% of the dry weight).

The iron is in a trivalent, polymerized form with the probable subunit structure of  $(\text{FeOOH}_8) \cdot (\text{FeO} \cdot \text{PO}_3\text{H}_2)$ .

The protein structure of ferritin appears to be organ specific. Hepatic ferritin differs from splenic ferritin in electrophoretic mobility and in tryptic peptide "fingerprint" pattern.<sup>321</sup> Furthermore, two distinct ferritins can be extracted from bone marrow.<sup>340</sup> One of these ("anabolic" ferritin) is of red cell origin, and its iron is derived from transferrin. The other ("catabolic" ferritin) is of RE origin, possibly identical to splenic ferritin, and derives its iron from hemoglobin. It seems likely that the various ferritins have functional as well as structural differences, but these remain to be demonstrated.

In a variety of organs, the synthesis of apoferritin is stimulated by exposure to iron, both *in vivo* and *in vitro*.<sup>319</sup> This phenomenon is blocked by inhibitors of protein synthesis (eg, cycloheximide), but not by inhibitors of RNA synthesis (eg, actinomycin D).<sup>319,371</sup> One would presume, therefore, that this effect of iron occurs at the translational level of protein synthesis. Possibly, accumulation of small amounts of apoferritin inhibits further polyribosomal synthesis, but when iron is added and ferritin is formed, the inhibition is released.

Two model mechanisms have been proposed for the combination of iron and apoferritin to form ferritin. In the conventional model, apoferritin is visualized as a pre-formed, hollow sphere with an external diameter of 12.0 nm and an internal diameter of 7.0 nm. Iron enters the sphere as ferrous ion; it is oxidized to the ferric form by molecular oxygen and is deposited irregularly and in variable amounts until saturation is achieved. This model is supported by x-ray diffraction studies.<sup>336</sup> In the second proposed model<sup>381</sup> the 7.0-nm iron core is pre-formed by polymerization of a ferric chelate. Then, apoferritin subunits form around this core to complete the molecule. The *in vitro* incorporation of transferrin iron into ferritin requires both a reducing agent, of which ascorbate is the most effective, and an iron chelating agent, such as ATP.<sup>372</sup> The reducing agent

probably functions, at least in part, to liberate iron from transferrin. The chelating agents may facilitate passage of iron through biologic membranes. Thus, these observations may or may not be applicable to situations in which the iron source is not transferrin, as in the gut mucosa or in the RE cell.

Two views also exist regarding the mechanism of mobilization of iron from ferritin. In the first, mobilization is considered to occur by reduction of the iron to the ferrous form, thereby releasing it from the protein. A variety of reducing agents can produce this effect *in vitro*. A possible *in vivo* reducing system, namely, xanthine oxidase, has been implicated in ferritin iron release in a series of studies by Mazur et al.<sup>370</sup> The second proposed mechanism postulates that iron is mobilized from ferritin by low molecular-weight chelating agents, without the necessity for reduction to the ferrous form.<sup>382</sup>

Hemosiderin was formerly distinguished from ferritin on the basis of solubility and the Prussian-blue reaction. Ferritin was considered to be soluble and Prussian-blue negative; hemosiderin, insoluble and Prussian-blue positive. Now that the ferritin molecule can be recognized by electron microscopy (page 33), these distinctions are no longer clear-cut. For example, a number of Prussian-blue positive, insoluble granules have been shown to contain ferritin.<sup>308,329,393</sup> On the basis of physical and chemical characteristics described in the previous paragraphs it is becoming clear that ferritin is a relatively homogeneous substance. By comparison, hemosiderin is a less precisely definable entity,<sup>390</sup> generally differing from ferritin in having a higher iron-to-protein ratio as well as being less soluble in aqueous solutions.

In most instances, hemosiderin appears to be derived from ferritin. Ferritin polymers of various sizes may represent intermediates between ferritin and recognizable forms of hemosiderin.<sup>409</sup> The simplest form of hemosiderin is a dense aggregate or crystal of ferritin, which may or may not be enclosed by a membrane.<sup>350</sup> It has been found that the proportion of iron in hemosiderin is variable (24 to 45%), but usually is much greater than

that in ferritin (16 to 23%). Thus, it seems likely that, in the formation of hemosiderin, protein is lost by denaturation or proteolysis. Morphologic evidence is consistent with this view.<sup>337</sup> As the protein is lost, the iron units acquire the characteristics of  $\text{Fe}_2\text{O}_3$  units. It has also been shown that structures that have the histochemical characteristics of hemosiderin may be very complex and contain lipids, porphyrins, and other substances in addition to iron.<sup>390</sup>

From a physiologic viewpoint, hemosiderin appears to represent a more stable and less available form of storage iron than ferritin.<sup>377,396</sup> Newly deposited or newly mobilized iron enters or leaves the ferritin compartment. Only after prolonged storage or continued mobilization does the hemosiderin compartment change in size.

#### Iron Metabolism in Other Tissues

Iron is required in small amounts by many tissues for the synthesis of non-hemoglobin, iron-containing enzymes and proteins, among which are the cytochromes, catalase and myoglobin. It is presumed that most of these tissues acquire their iron from transferrin<sup>359</sup> and there is indirect evidence for the existence of receptor sites analogous to those on erythrocytes.<sup>338</sup> Of the non-erythrocyte iron pathways, the exchange with the *hepatic parenchymal cell* seems to be the largest and has been the most extensively studied. Normally, about 5% of iron leaving plasma is accounted for by this pathway.<sup>335,359</sup> This fraction increases greatly when transferrin saturation increases or when erythropoiesis is depressed. The *placenta* also accepts iron from transferrin, and may do so even at relatively low plasma iron levels, thus effectively competing with the maternal erythroid marrow.<sup>359,365</sup> This function appears to be independent of fetal influences and is a property of the placental cell itself.<sup>368</sup> Under normal circumstances, efficient transfer to the fetus occurs, but some iron is stored as ferritin and hemosiderin. The *gut* also accepts iron from transferrin, and this pathway may have a regulatory function in iron absorption (page 157).

*Skeletal muscle* utilizes iron for myoglobin synthesis and also maintains an iron storage pool that, although large, is not easily mobilized for use by other tissues.<sup>324</sup>

#### Ferrokinetics<sup>311,335,394,385</sup>

It is possible to measure some of the rates at which iron moves around the iron cycle by using radioactive tracers. In the usual ferrokinetic study, transferrin is labeled with <sup>59</sup>Fe. The tracer is then monitored as it moves from the plasma to the bone marrow and into the circulating red cells. Since the kinetics of iron are intimately related to hemoglobin synthesis, such a study makes it possible to assess rates and sites of erythropoiesis, and to evaluate "ineffective" and "effective" erythropoiesis (Chapter 13).

<sup>59</sup>Fe is injected directly as ferrous citrate or is first bound to transferrin by incubation with fresh plasma. Serial samples of blood are taken at frequent intervals during the first several hours and daily thereafter. These are analyzed for plasma and red cell radioactivity, plasma iron concentration, and VPRC. For some calculations, a separate determination of plasma volume is made (page 121), or it may be calculated from the extrapolated zero-time plasma radioactivity and the total injected radioactivity. The basic measurements calculated from these data are given in Table 4-5.

The plasma iron transport rate (PIT) is a measure of the rate at which iron leaves the plasma. It may be expressed either as a total daily rate (ie, mg of iron per day) or as a rate per volume (dl) of blood. The latter mode of expression has the two advantages of obviating the need for determining plasma volume and of incorporating a built-in correction for body size. It assumes, however, that the blood volume is approximately normal and should not be used if there are clinical reasons to doubt this assumption. The PIT is generally considered to be a good index of "total" erythropoiesis, whether "effective" or "ineffective." It correlates well with the total nucleated red cell mass and with the rate of red cell production induced by phlebotomy.<sup>335</sup> However, when erythro-

Table 4-5. Basic Ferrokinetic Measurements

Measurement	Calculation	Average Normal Value
$t_{1/2}$	Graphically, from semilogarithmic plot of plasma radioactivity disappearance	86 minutes
PIT	$\frac{0.693}{t_{1/2}} \times \text{plasma Fe (mg/ml)} \times \text{plasma vol (ml)} \times 1440 \text{ min/day}$	26 mg/day
	-or-	-or-
	$\frac{\text{plasma Fe } (\mu\text{g/dl}) \times 100 - \text{VPRC}}{t_{1/2} \times 100}$	0.7 mg/day/dl blood
RCU	$\frac{14\text{-day radioactivity/ml blood} \times 100}{0 \text{ time radioactivity/ml blood}}$	80%
	-or-	
	$\frac{14\text{-day radioactivity/ml RBC} \times \text{red cell mass (ml)} \times 100}{\text{total injected radioactivity}}$	
EIT	$\text{PIT} \times \text{RCU}$	21 mg/day
		-or-
		0.56 mg/day/dl blood
MTT	Graphically, from a semilogarithmic plot the time at which $100 - \text{RCU} = 50\%$	3.5 days

Abbreviations  $t_{1/2}$ , plasma Fe half-disappearance time; PIT, plasma iron transport rate; RCU, red blood cell utilization; EIT, erythrocyte iron turnover rate; MTT, marrow transit time; VPRC, volume of packed red cells.

poiesis is reduced, or when the degree of transferrin saturation is high, the interpretation of PIT is complicated by transfer of iron to tissues other than marrow.

The erythrocyte iron turnover rate (EIT) is a measure of the rate at which iron moves from marrow to circulating red cells. Like PIT, it may be expressed as a total daily rate or as a rate per dl of blood, and the same advantages and disadvantages of the two modes of expression apply. It is an index of "effective" erythropoiesis and correlates well with an appropriately corrected reticulocyte index<sup>389</sup> (Chapter 20, page 731).

The observation that red cell iron utilization (RCU) is usually less than 100%, and that there is, therefore, a discrepancy between PIT and EIT, indicates that a proportion of the iron leaving plasma does not make its appearance in circulating red cells. There are several possible fates for this "nonutilized" iron. It may enter non-erythroid tissues; it may be lost with hemoglobin when normoblast denudation occurs; it may be lost with intramedullary destruction of defective red cells ("ineffective" erythropoiesis); and, fi-

nally, it may enter one of several "reflux" pathways, to be discussed further below. When RCU is reduced and there is a relatively large difference between PIT and EIT, one of these pathways accounts for a larger than normal proportion of plasma iron turnover.

The marrow transit time (MTT) may be used to evaluate erythropoietin response. In general, this value decreases in proportion to the degree of erythropoietic stimulation. In situations characterized by an appropriate marrow response to anemia, there is a predictable relation between VPRC and MTT (Fig. 4-9).

In addition to the above determinations on blood specimens, it is possible to monitor iron movements within the body by surface counting over the liver, spleen, and sacrum (marrow). This procedure may be particularly useful in detecting extramedullary hematopoiesis.

Variations of ferrokinetic measurements in representative disease states are illustrated in Figure 4-10 and Table 4-6. Ferrokinetic patterns in other disorders are discussed in the

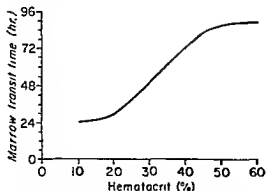


Fig 4-9 Relation of marrow transit time to volume of packed red cells (hematocrit) in normal subjects (From Finch et al.<sup>335</sup> courtesy of the authors and Medicine)

chapters dealing with specific disease entities. When erythropoiesis is reduced, as in hypoplastic anemia, the PIT may be normal or only slightly reduced, but RCU and EIT are greatly reduced. Furthermore, iron appears early in the liver and is retained there. When erythropoiesis is accelerated in hemolytic anemia, both PIT and EIT are increased and

radioactivity accumulates at the sites of destruction, ie, the spleen or liver. In states of ineffective erythropoiesis, eg, thalassemia major, PIT is greatly increased, RCU is reduced, and EIT is relatively normal. There may be early iron uptake over organs in which the defective cells are destroyed.

The above ferrokinetic measurements are useful for clinical and investigational purposes, but they must be considered only approximations of the parameters that they purport to measure. The calculations are based on the incorrect assumption that iron leaves plasma at a single, exponential rate. The early part of the plasma disappearance curve does, in fact, approximate a straight line on semilogarithmic paper. However, after several hours, there is a significant change in the slope of the line until another, much slower, exponential disappearance rate is established (Fig. 4-11). This phenomenon is explained by the return to plasma of about 35% of the iron that leaves it.<sup>335</sup> To account for this reflux, various models have been

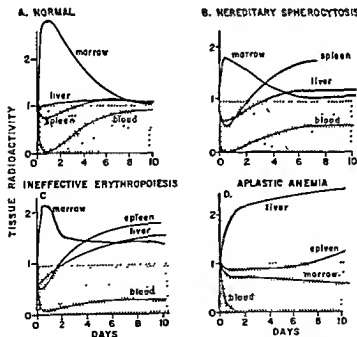


Fig 4-10 Pattern of radioactivity in blood and over sacrum (marrow), liver, and spleen during a ferrokinetic study. Representative data from patients with four conditions are presented. Compare with Table 4-6 (From Finch et al.<sup>335</sup> courtesy of the authors and Medicine)

**Table 4-6. Ferrokinetic Data in Representative Clinical Situations**<sup>310,335</sup>

	$T_{1/2}$ (min)	$PIT$ (mg/day/dl)	$RCU$ (%)	$EIT$ (mg/day/dl)
Normal	86	0.7	80	0.56
Hypoplastic anemia	267	0.45	23	0.10
Hemolytic anemia (hereditary spherocytosis)	24	3.42	57	1.87
Ineffective erythropoiesis (thalassemia major)	21	6.87	18	1.24

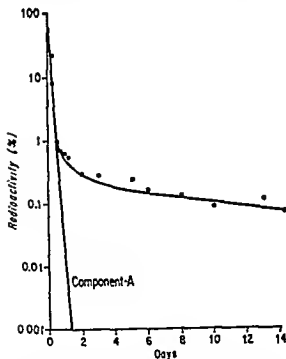
Values are means

proposed, of which the most widely used has been that of Pollycove and associates.<sup>385</sup> Central to their model is a proposed "labile iron pool," about 80 mg in size, that is interposed between the plasma and red cell pools and from which reflux of iron to plasma occurs. It has been impossible to demonstrate the existence of such a pool, however, and formidable evidence against the model has

been presented.<sup>335</sup> The reflux may be divided into two components, a rapid reflux of 5 to 10% with a  $t_{1/2}$  of about eight hours and a slower reflux of 25% of about eight days. The first may represent the exchange of transferrin with the extravascular space, and the second is probably a by-product of erythropoiesis. It is possible to correct the various ferrokinetic measures for reflux and thereby improve their accuracy. As yet, however, such correction factors have not been employed routinely, and the improved accuracy may not be necessary for most clinical purposes.

## Hemoglobin Structure and Synthesis

Hemoglobin is the name applied to the oxygen transport protein of erythrocytes. Since it accounts for about 90% of the dry weight of the mature erythrocyte, a major proportion of the biosynthetic activity of the developing normoblast must be devoted to its production. Hemoglobin is a conjugated protein with a molecular weight near 64,500. About 96% of the molecule is protein (globin) and the remainder is a highly colored prosthetic group, heme, a complex of iron and protoporphyrin (Fig. 4-12). An optimal rate of hemoglobin synthesis requires the proper functioning of three complex metabolic pathways, corresponding to each of the three structural components of hemoglobin. One of these, the iron metabolic pathway, has been



**Fig. 4-11.** The plasma radioiron disappearance curve. The curve can be resolved into two components. The initial, rapid disappearance (component A) is used for routine ferrokinetic calculations. The second, slower component results from several reflux pathways described in the text. (From Finch et al.<sup>335</sup> courtesy of the authors and Medicine)



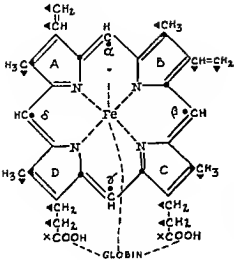


Fig. 4-12. Chemical structure of heme and its manner of union with globin to form hemoglobin. The carbon atoms derived from the alpha carbon of glycine are represented by • those supplied from the methyl carbon of acetate by A, those derived from the carboxyl group of acetate by x. The unmarked carbons are those which are derived from either the methyl carbon atom of acetate or from the carboxyl atom (Prepared by Dr. G. E. Cartwright.)

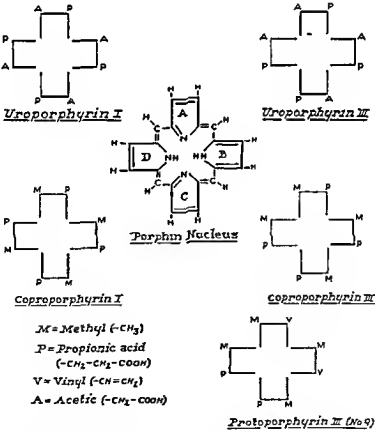


Fig. 4-13. Structural formula of the porphyrin nucleus and diagrammatic representation of some naturally occurring porphyrins (Prepared by Dr. G. E. Cartwright.)

discussed on preceding pages. The pathways for the biosynthesis of heme and of globin will be discussed in this section.

## Heme

In order to understand heme structure and synthesis, the terminology applied to the porphyrins must be appreciated.<sup>447-463</sup> Porphyrins are tetrapyrroles. The four pyrrole rings are united by four methene ( $=C-$ ) bridges, thus forming the porphyrin ring. This is a resonant structure and is characterized by distinctive properties of color and fluorescence. The simplest porphyrin, porphin (Fig. 4-13), does not occur in nature; in the natural compounds, eight of the hydrogen atoms have been replaced by certain substituent groups, namely, methyl ( $-CH_3$ ), vinyl ( $-CH=CH_2$ ), acetic acid ( $-CH_2-COOH$ ), or propionic acid ( $-CH_2-CH_2-COOH$ ). The porphyrin in hemoglobin, protoporphyrin, has only two carboxyl ( $-COOH$ ) groups. In protoporphyrin, eight hydrogen atoms of the porphin nucleus are replaced by four methyl, two vinyl, and two propionic acid groups (Fig. 4-13). Normal feces contain small amounts of a tetra-carboxylated porphyrin, which Fischer called coproporphyrin. Both coproporphyrin and protoporphyrin are soluble in nonpolar solvents, but are relatively insoluble in water at physiologic pH. The third important porphyrin compound is uroporphyrin, so named because it was first isolated from urine. Uroporphyrin differs from coproporphyrin in that the four methyl groups are replaced by acetic acid side chains (Fig. 4-13). This octa-carboxylated acid is water soluble and is insoluble in ether and most other organic solvents.

In theory, there are four possible ways in which the two types of side chains in coproporphyrin and uroporphyrin can be arranged. The isomers that result are designated by roman numerals, I, II, III, and IV. Only types I and III are found in nature, and only type III porphyrins can be utilized for heme synthesis. In type I porphyrins the substituent groups alternate regularly, as shown in

Figure 4-13; in type III, one pair is asymmetrical. When three types of side chains are present, as in protoporphyrin, the number of possible isomers is 15. The protoporphyrin isomer in hemoglobin is designated protoporphyrin 9, type III.

The propionic acid side chains of protoporphyrin may possibly function in the ionized form to orient the heme and help attach it to globin. The methyl groups protect the positions at which they are attached. The vinyl groups are required to make possible the insertion of iron into the porphyrin ring.<sup>451,456</sup>

## Biosynthesis of Heme<sup>451,465,467</sup> (Fig. 4-14)

From isotope studies *in vivo*,<sup>487</sup> by incubation of labeled precursors with avian erythrocytes<sup>479</sup> and mammalian reticulocytes,<sup>460</sup> and from studies of the microorganism, *Rhodospseudomonas spheroides*,<sup>465</sup> a clear picture has been gained concerning steps in the synthesis of heme. The first precursor of heme,  $\delta$ -aminolevulinic acid (ALA), is derived from the condensation of the 2-carbon amino acid, glycine, and "activated" succinic acid (succinyl CoA).<sup>459</sup> The latter can be formed in several ways. In the tricarboxylic acid (Krebs) cycle, its normal precursor is  $\alpha$ -ketoglutarate. Other metabolic sources of succinyl CoA include (1) a reaction of succinate with CoA and guanosine triphosphate in the presence of the enzyme succinyl CoA synthetase; (2) the transfer of CoA from acetoacetyl CoA to succinate, a reaction catalyzed by acetoacetyl CoA: succinyl CoA transferase; and (3) valine and isoleucine, which are metabolized to succinyl CoA via propionyl CoA and methylmalonyl CoA.<sup>446</sup> The relative importance of these various sources in heme biosynthesis remains to be determined.

The condensation of glycine with succinyl CoA takes place in the presence of the enzyme, ALA synthetase.<sup>448,464</sup> The level of this enzyme, which is the most important rate-limiting factor in the heme biosynthetic pathway, is controlled by induction and repression of enzyme synthesis.<sup>450,455</sup> Certain

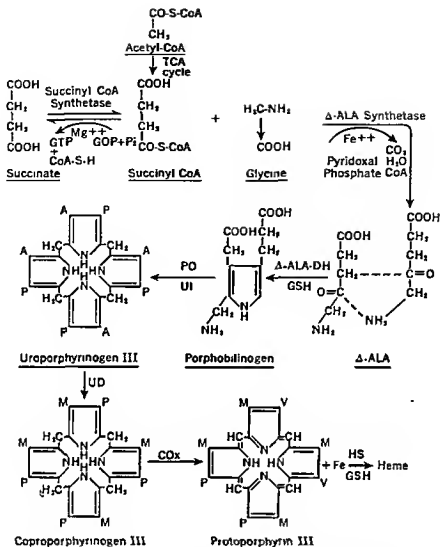


Fig 4-14 Chemical steps in the biosynthesis of heme. For abbreviations see text

chemicals, such as allylisopropylacetamide, bring about excessive enzyme induction, thereby causing a type of porphyria. Perhaps of more physiologic significance is the observation that certain sex hormones and their derivatives may behave as inducers.<sup>450,468</sup> Glucose and high-carbohydrate diets block induction of the enzyme,<sup>482</sup> perhaps by promoting conjugation of the inducers with glucuronide,<sup>450</sup> and hence may be used in therapy of porphyria (Chapter 32). Heme, the end product of the pathway, represses synthesis of ALA synthetase.<sup>458</sup> Another factor that affects the rate of this reaction is feedback

inhibition<sup>444</sup>; heme not only represses enzyme synthesis but also inhibits the activity of enzyme already formed. Heme appears to accomplish this inhibition by forming a coordination complex between its iron atom and the enzyme.<sup>470</sup> Thus, because of its repressive and inhibitory effects, any accumulation of heme leads to a reduced rate of ALA synthesis. Conversely, if heme is removed rapidly by union with globin, ALA synthesis is stimulated.

It is possible that the first product of the ALA synthetase reaction is  $\alpha$ -amino- $\beta$ -keto-adipate,<sup>478</sup> an unstable compound that is rap-

idly decarboxylated to ALA. Synthesis of ALA requires the participation of two vitamins, pyridoxine, in the form of pyridoxal phosphate, and pantothenic acid, as a component of CoA.<sup>474</sup> It has been suggested that iron is also a cofactor in the reaction<sup>443</sup>; however, other observations indicate that the effect of iron is explained by augmentation of enzyme synthesis.<sup>481</sup>

In the next step, two ALA molecules are joined in the presence of the enzyme,  $\delta$ -aminolevulinic dehydrase ( $\Delta$ -ALA-DH),<sup>448,485</sup> to form porphobilinogen (PBG), a monopyrrole that carries acetic acid and propionic acid side chains.<sup>452</sup> The enzyme requires sulfhydryl groups for its activity and therefore is inhibited by lead and stabilized by glutathione.

In the succeeding step, four PBG molecules condense to form uroporphyrinogen III.<sup>442,453,466</sup> This reaction requires two enzymes, uroporphyrinogen synthetase (porphobilinogen deaminase, PD) and uroporphyrinogen III cosynthetase (uroporphyrinogen isomerase, UI). Many mechanisms have been proposed to explain the manner in which uroporphyrinogen III is formed.<sup>472</sup> It is probable that, in the presence of uroporphyrinogen synthetase, PBG is converted to an intermediate compound. In the absence of uroporphyrinogen III cosynthetase, this intermediate spontaneously becomes converted to uroporphyrinogen I, but, in the presence of the cosynthetase, uroporphyrinogen III is formed. Several structures for the hypothetical intermediate have been proposed,<sup>442,467,472,486</sup> but none has been proved.

Uroporphyrinogen and other porphyrinogens differ chemically from their corresponding porphyrins in that they have six additional hydrogen atoms in the tetrapyrrole ring (compare Figs. 4-13 and 4-14). With this alteration, the ring does not resonate, and therefore the porphyrinogens are colorless and do not fluoresce. In the presence of light and oxygen, spontaneous oxidation of porphyrinogens to porphyrins occurs. Since porphyrins cannot be utilized in heme synthesis, the porphyrinogens are considered to be the

true metabolic intermediates in the pathway.<sup>442</sup>

Coproporphyrinogen III is formed from uroporphyrinogen III by decarboxylation of the four acetic acid side chains. The conversion is catalyzed by the enzyme uroporphyrinogen decarboxylase (UD).<sup>452,453,469</sup> It is probable that the four carboxyl groups are removed sequentially and that seven, six, and five carboxyl porphyrinogens are intermediates in the reaction.

Conversion of coproporphyrinogen III to protoporphyrin requires a mitochondrial enzyme (or system of enzymes) called coproporphyrinogen oxidase (COX) or coproporphyrinogenase.<sup>441,451,454,473</sup> In mammalian tissues and aerobic bacteria, the reaction requires molecular oxygen as a hydrogen acceptor, and it is probable that a tricarboxylic porphyrinogen and protoporphyrinogen are reaction intermediates.

The last step in heme synthesis is catalyzed by an enzyme, called heme synthetase<sup>449,456,475</sup> or ferrochelatase,<sup>462,477</sup> that is located on the inner mitochondrial membrane.<sup>455</sup> In this step, ferrous iron is inserted into the protoporphyrin ring. The reaction is enhanced *in vitro* by reducing agents, such as cysteine, glutathione, or ascorbate, that apparently act by maintaining the iron in the reduced state. Possibly because of the opposite effect, the reaction is inhibited by aerobic conditions. A phospholipid may be a cofactor in the reaction.<sup>456</sup>

The biosynthesis of heme bears a peculiar relation to mitochondria. The first step and the last two steps are mitochondrial, whereas the intermediate steps occur in the extramitochondrial cytoplasm. Consequently, as they mature and their mitochondria degenerate, erythrocytes lose the ability to form ALA and to convert coproporphyrinogen III to heme. However, they retain the ability to convert ALA to coproporphyrinogen.

#### Porphyrins in Erythrocytes, Urine, and Stools<sup>447,477</sup>

In red cells, in addition to the protoporphyrin incorporated into heme, free proto-

porphyrin (FEP) and free coproporphyrin (FEC) are found in small amounts.<sup>476</sup> (For normal values, see Appendix, Table A-7.) The concentration of FEP increases when protoporphyrin is not utilized efficiently for heme synthesis,<sup>475</sup> as in iron deficiency,<sup>463</sup> the anemia of chronic disorders, lead intoxication, and certain sideroblastic anemias<sup>461</sup> (Chapters 16, 17, and 18). In persons with untreated pernicious anemia, low or low normal values are found. The concentration of FEC correlates with the reticulocyte count.<sup>476,483</sup> Thus, in hemolytic anemia, a relatively marked increase in FEC occurs although FEP is only moderately elevated.

Coproporphyrin and uroporphyrin, formed as by-products of heme synthesis, are excreted into the urine and stools.<sup>457,477</sup> (For normal values see Appendix, Table A-8.) Approximately half of the freshly passed porphyrins are excreted as the porphyrinogen. Increased urinary coproporphyrin may be found in association with lead poisoning, liver disease, and hemolytic anemia.<sup>484</sup> Fecal coproporphyrin is increased in patients with hemolytic anemia and decreased in those with liver disease. The ratio of urinary to fecal excretion of coproporphyrin appears to be related to hepatic function.

The porphyrias are a group of disorders in which great excesses of porphyrins and porphyrin precursors are excreted. These diseases are discussed in Chapter 32.

### Globin

From the standpoint of protein structure, there are several normal hemoglobins. Hemoglobin A or adult hemoglobin comprises 96 to 98% of the hemoglobin in adults. Most of the remainder is a structurally different hemoglobin called hemoglobin A<sub>2</sub>. In fetal life and in early infancy the predominant hemoglobin is fetal hemoglobin or hemoglobin F. During the first year of life, hemoglobin F is gradually replaced by hemoglobins A and A<sub>2</sub>, so that the former constitutes less than 1% of the hemoglobin of adults. Other normal hemoglobins (Gower I, II) are found only in the embryo (Chapter 2).<sup>508</sup>

All of the normal hemoglobins contain two pairs of polypeptide chains, and to each chain is attached one heme group. In hemoglobin A, the polypeptide chains of one pair are designated *alpha* ( $\alpha^A$ ) chains, and those of the other pair are called *beta* ( $\beta^A$ ) chains. This structure is signified by the formula,  $\alpha_2^A\beta_2^A$ . Fetal hemoglobin contains a pair of  $\alpha^A$ -chains identical to those found in hemoglobin A, but the chains of the second pair differ and are called *gamma* ( $\gamma^F$ ) chains; thus, hemoglobin F is designated  $\alpha_2^A\gamma_2^F$ . Hemoglobin A<sub>2</sub> contains a pair of  $\alpha$ -chains and a pair of *delta* ( $\delta^{A_2}$ ) chains and is designated  $\alpha_2^A\delta_2^{A_2}$ . The embryonic hemoglobin, Gower II, has the structure  $\alpha_2^E\zeta_2$ . Hemoglobin A<sub>2</sub> can be separated from hemoglobins A and F and quantified by means of a variety of electrophoretic or chromatographic procedures.<sup>504,516,523</sup> Many routine electrophoretic procedures do not distinguish hemoglobin F from A, but separation can be accomplished at pH 6.0 in agar gel.<sup>521</sup> More commonly, hemoglobin F is detected and quantified by its ability to resist denaturation in alkaline reagents (the "alkaline denaturation test," page 808).

Additional heterogeneity of normal hemoglobin has been observed. A fast-moving fraction (hemoglobin A<sub>3</sub>) that is detected by starch block electrophoresis is probably the same as fraction A<sub>1</sub> found on chromatography<sup>524</sup> (Fig. 4-15). These fractions contain a mixture of hemoglobins, of which some appear to be artifacts of storage or preparation, but others occur in vivo and may change in concentration with disease.<sup>509</sup> Although the exact nature of all of these hemoglobins is not known, most appear to be due to acquired alterations in the  $\beta^A$ -chain.<sup>501,509</sup> Still further heterogeneity results from genetic variations in hemoglobin structure, as discussed in Chapter 24.

### Structure of Globin<sup>513</sup>

The  $\alpha^A$ -,  $\beta^A$ -,  $\gamma^F$ -, and  $\delta^{A_2}$ -chains differ from one another in protein structure. In describing this structure, use will be made of a terminology that recognizes four different aspects of structure. These are: (1) primary

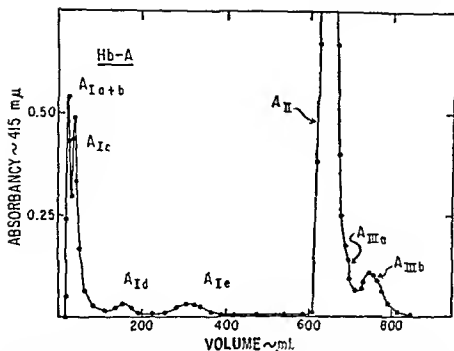


Fig. 4-15 Column chromatography of hemoglobin A on IRC-50 (method of Clegg and Schroeder, Developer No 5 used). The main component  $A_{III}$  corresponds to the Hb- $A_1$  demonstrated by starch-block electrophoresis.  $A_{IIIc}$  corresponds to Hb- $A_2$ , and  $A_{II}$  corresponds to  $A_3$ . (Courtesy of Dr. Robert L. Hill)

structure, or the sequence of amino acids, (2) secondary structure, which describes the organization of amino acids into helices stabilized by hydrogen bonds between the CO and NH groups of adjacent coils,<sup>517</sup> (3) tertiary structure, which refers to the manner in which the polypeptide chains are folded to form a three-dimensional, spherical unit stabilized by hydrophobic Van der Waals forces, and (4) quaternary structure, or the way in which several polypeptide chains join to form a single molecule.

It is now possible to state the exact primary structure of all four normal hemoglobin chains<sup>513</sup> (Table 4-7). The elucidation of this complex information required the application of a variety of sophisticated biochemical maneuvers that can be described here only superficially (for methodologic details, see ref 506). First, the chains were separated from one another in 8M urea solutions and isolated by counter-current distribution or chromatography. Then they were digested with trypsin to form a series of smaller peptides. These in turn were isolated by peptide-mapping ("fingerprinting") or by chroma-

tography (Chapter 24, page 810). Finally, the amino acid sequence of each peptide was determined chemically.

In spite of the differences in the primary structure of the chains, their secondary structures are remarkably similar. There are eight helical segments in each, and these helices are designated by the letters A through H<sup>519</sup> (Table 4-7). The helices are of nearly identical length in all four normal chains except for the D helix, which contains seven amino acids in the  $\beta$ -,  $\gamma$ -, and  $\delta$ -chains, but only two amino acids in the  $\alpha$ -chain. The helices make up about 75% of the molecule. Interspersed between them are seven non-helical segments: NA, AB, CD, EF, FG, GH, and HC. This arrangement is important structurally because the helices are relatively rigid and linear whereas the non-helical segments allow bending.

A given amino acid in a polypeptide chain may be denoted either by its sequential number or by a helical number. In the sequential system, the N-terminal amino acid is assigned the number 1, and each succeeding amino acid receives the next higher number until the

Table 4-7. Primary and Secondary Structure of Hemoglobin Polypeptide Chains

Helix #	Amino Acid Sequence					
	#	$\alpha$	$\beta$	$\gamma$	$\delta$	#
NA1	1	Val	Val	Gly	Val	1
NA2	2	Leu	His	His	His	2
NA3			Leu	Phe	Leu	3
A1	3	Ser	Thr	Thr	Thr	4
A2	4	Pro	Pro	Glu	Pro	5
A3	5	Ala	Glu	Glu	Glu	6
A4	6	Asp	Glu	Asp	Glu	7
A5	7	Lys	Lys	Lys	Lys	8
A6	8	Thr	Ser	Ala	Thr	9
A7	9	Asn	Ala	Thr	Ala	10
A8	10	Val	Val	Ile	Val	11
A9	11	Lys	Thr	Thr	Asn	12
A10	12	Ala	Ala	Ser	Ala	13
A11	13	Ala	Leu	Leu	Leu	14
A12	14	Try	Try	Try	Try	15
A13	15	Gly	Gly	Gly	Gly	16
A14	16	Lys	Lys	Lys	Lys	17
A15	17	Val	Val	Val	Val	18
A16	18	Gly				
AB1	19	Ala				
B1	20	His	Asn	Asn	Asn	19
B2	21	Ala	Val	Val	Val	20
B3	22	Gly	Asp	Glu	Asp	21
B4	23	Glu	Glu	Asp	Ala	22
B6	24	Tyr	Val	Ala	Val	23
B8	26	Gly	Gly	Gly	Gly	24
B7	26	Ala	Gly	Gly	Gly	25
B8	27	Glu	Glu	Glu	Glu	26
B8	28	Ala	Ala	Thr	Ala	27
B10	29	Leu	Leu	Leu	Leu	28
B11	30	Glu	Gly	Gly	Gly	29
B12	31	Arg	Arg	Arg	Arg	30
B13	32	Met	Leu	Leu	Leu	31
B14	33	Leu	Leu	Leu	Leu	32
B15	34	Leu	Val	Val	Val	33
B16	35	Ser	Val	Val	Val	34
C1	36	Phe	Tyr	Tyr	Tyr	35
C2	37	Pro	Pro	Pro	Pro	36
C3	38	Thr	Try	Try	Try	37
C4	39	Thr	Thr	Thr	Thr	38
C5	40	Lys	Gln	Gln	Gln	39
C6	41	Thr	Arg	Arg	Arg	40
C7	42	Tyr	Phe	Phe	Phe	41
CD1	43	Phe	Phe	Phe	Phe	42
CD2	44	Pro	Glu	Asp	Glu	43
CD3	45	His	Ser	Ser	Ser	44
CD4	46	Phe	Phe	Phe	Phe	45
CD5	47	Asp	Gly	Gly	Gly	46
CD6	48	Leu	Asp	Asn	Asp	47
CD7	49	Ser	Leu	Leu	Leu	48
CD8			Ser	Ser	Ser	49
D1	50	His	Thr	Ser	Ser	50
D2	51	Gly	Pro	Ala	Pro	51
D3			Asp	Ser	Asp	52
D4			Ala	Ala	Ala	53
D5			Val	Ile	Val	54
D6			Met	Met	Met	55
D7			Gly	Gly	Gly	56
E1	52	Ser	Asn	Asn	Asn	57
E2	53	Ala	Pro	Pro	Pro	58
E3	54	Gln	Lys	Lys	Lys	59
E4	55	Val	Val	Val	Val	60
E5	56	Lys	Lys	Lys	Lys	61
E6	57	Gly	Ala	Ala	Ala	62
E7	58	His	His	His	His	63
E8	59	Gly	Gly	Gly	Gly	64
E9	60	Lys	Lys	Lys	Lys	65
E10	61	Lys	Lys	Lys	Lys	66
E11	62	Val	Val	Val	Val	67
E12	63	Ala	Leu	Leu	Leu	68
E13	64	Asp	Gly	Thr	Gly	69
E14	65	Ala	Ala	Ser	Ala	70
E15	66	Leu	Phe	Leu	Phe	71
E16	67	Thr	Ser	Gly	Ser	72
E17	68	Asn	Asp	Asp	Asp	73
E18	69	Ala	Gly	Ala	Gly	74
E19	70	Val	Leu	Ile	Leu	75
E20	71	Ala	Ala	Lys	Ala	76
EF1	72	His	His	His	His	77
EF2	73	Val	Leu	Leu	Leu	78
EF3	74	Asp	Asp	Asp	Asp	79
EF4	75	Asp	Asn	Asp	Asn	80
EF5	76	Met	Leu	Leu	Leu	81
EF6	77	Pro	Lys	Lys	Lys	82
EF7	78	Asn	Gly	Gly	Gly	83
EF8	79	Ala	Thr	Thr	Thr	84
F1	80	Leu	Phe	Phe	Phe	85
F2	81	Ser	Ala	Ala	Ser	86
F3	82	Ala	Thr	Gln	Gln	87
F4	83	Leu	Leu	Leu	Leu	88
F5	84	Ser	Ser	Ser	Ser	89
F6	85	Asp	Glu	Glu	Glu	90
F7	86	Leu	Leu	Leu	Leu	91
F8	87	His	His	His	His	92
F9	88	Ala	Cys	Cys	Cys	93
FG1	89	His	Asp	Asp	Asp	94
FG2	90	Lys	Lys	Lys	Lys	95
FG3	91	Leu	Leu	Leu	Leu	96
FG4	92	Arg	His	His	His	97
FG5	93	Val	Val	Val	Val	98

\*Ala at H14 (136) in the  $\gamma$ - and  $\delta$ -chains may be normal variants

Amino acids are indicated by a three-letter code. Uncharged amino acids: Gly, glycine, Ala, alanine, Val, valine, Leu, leucine, Ile, isoleucine, Gln, glutamine, Asn, asparagine, Met, methionine, Cys, cystine, Phe, phenylalanine, Tyr, tyrosine, Thr, threonine, Pro, proline, Ser, serine, Thr, threonine. Charged amino acids: Asp, aspartic acid (-1), Glu, glutamic acid (-1), Arg, arginine (+1), Lys, lysine (+1), His, histidine (+1).

Table 4-7. Continued.

Helix #	Amino Acid Sequence					
	#	$\alpha$	$\beta$	$\gamma$	$\delta$	#
G1	94	Asp	Asp	Asp	Asp	99
G2	95	Pro	Pro	Pro	Pro	100
G3	96	Val	Glu	Glu	Glu	101
G4	97	Asn	Asn	Asn	Asn	102
G5	98	Phe	Phe	Phe	Phe	103
G6	99	Lys	Arg	Lys	Arg	104
G7	100	Leu	Leu	Leu	Leu	105
G8	101	Leu	Leu	Leu	Leu	106
G9	102	Ser	Gly	Gly	Gly	107
G10	103	His	Asn	Asn	Asn	108
G11	104	Cys	Val	Val	Val	109
G12	105	Leu	Leu	Leu	Leu	110
G13	106	Leu	Val	Val	Val	111
G14	107	Val	Cys	Thr	Cys	112
G15	108	Thr	Val	Val	Val	113
G16	109	Leu	Leu	Leu	Leu	114
G17	110	Ala	Ala	Ala	Ala	115
G18	111	Ala	His	Ile	Arg	116
G19	112	His	His	His	Asn	117
GH1	113	Leu	Phe	Phe	Phe	118
GH2	114	Pro	Gly	Gly	Gly	119
GH3	115	Ala	Lys	Lys	Lys	120
GH4	116	Glu	Glu	Glu	Glu	121
GH6	117	Phe	Phe	Phe	Phe	122
H1	118	Thr	Thr	Thr	Thr	123
H2	119	Pro	Pro	Pro	Pro	124
H3	120	Ala	Pro	Glu	Gln	125
H4	121	Val	Val	Val	Met	126
H5	122	His	Gln	Gln	Gln	127
H8	123	Ala	Ala	Ala	Ala	128
H7	124	Ser	Ala	Ser	Ala	129
H8	125	Leu	Tyr	Try	Tyr	130
H9	126	Asp	Gln	Gln	Gln	131
H10	127	Lys	Lys	Lys	Lys	132
H11	128	Phe	Val	Met	Val	133
H12	129	Leu	Val	Val	Val	134
H13	130	Ala	Ala	Thr	Ala	135
H14	131	Ser	Gly	Gly	Gly	136
H15	132	Val	Val	Val	Val	137
H16	133	Ser	Ala	Ala	Ala	138
H17	134	Thr	Asn	Ser	Asn	139
H18	135	Val	Ala	Ala	Ala	140
H19	136	Leu	Leu	Leu	Leu	141
H20	137	Thr	Ala	Ser	Ala	142
H21	138	Ser	His	Ser	His	143
HC1	139	Lys	Lys	Arg	Lys	144
HC2	140	Tyr	Tyr	Tyr	Tyr	145
HC3	141	Arg	His	His	His	146

C-terminal end is reached. With this system, amino acids are numbered from 1 to 141 in the  $\alpha$ -chain and from 1 to 146 in the  $\beta$ -,  $\gamma$ -, and  $\delta$ -chains. In the newer helical system, each amino acid is designated by a letter and a number which indicate the helix and the position in the helix, respectively. The helical system is gradually gaining favor because it illustrates the homology between chains and has more structural significance. For example, the histidine to which heme attaches is amino acid #87 in the  $\alpha$ -chain and #92 in the  $\beta$ -,  $\gamma$ -, and  $\delta$ -chains; the helical designation for this histidine is the same in all the normal chains, namely, F8.

The tertiary and quaternary structures of hemoglobin have been studied by x-ray diffraction techniques, especially by Perutz and his coworkers.<sup>519</sup> In aqueous solutions and in crystals, the polypeptide chains assume a structure in which the polar amino acids are presented to the molecular surface where they interact with water, rendering the molecule soluble. The groups directed toward the inner core of the molecule are all nonpolar, and the hydrophobic (Van der Waals) bonding that occurs between them makes the structure stable. The resulting, roughly spherical, tertiary structure is similar for all the normal hemoglobin polypeptides (Fig. 4-16) as well as for certain other heme proteins, such as myoglobin.

Heme iron forms a covalent bond with histidine at F8 (sometimes referred to as the "proximal" histidine). When oxygen is bound, it forms covalent bonds with heme and the histidine at E7 (the "distal" histidine). Thus, heme is suspended in a nonpolar crevice between the E and F helices (Fig. 4-16), but, in addition, it forms Van der Waals bonds with many other parts of the molecule and in this way makes an important contribution to tertiary structure. If heme is extracted, the central helical regions, C, D, E, and F, unfold with consequent decrease in solubility.<sup>507</sup> The nonpolar environment of the heme group makes it possible for the iron to form a reversible bond with oxygen without becoming oxidized to the ferric form.

When four polypeptide chains combine to



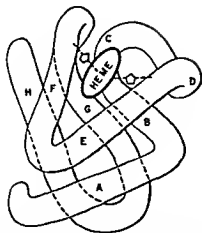


Fig. 4-16 The tertiary structure of a single globin polypeptide chain. The helical segments labeled A through H are relatively linear; bending of the chains occurs between helices. Heme is suspended in a crevice between the E and F helices. (Courtesy of C. A. Finch.)

form the complete hemoglobin molecule, each chain lies approximately at the vertices of a regular tetrahedron. A spherical molecule, about 6.0 nm in diameter, is formed. With high resolution x-ray diffraction, the nature of the contacts between chains has been explored in detail for horse hemoglobin.<sup>519</sup> Contacts between like chains, i.e.,  $\alpha_1\alpha_2$  and  $\beta_1\beta_2$ , are limited and of little importance. The two major contacts between unlike chains have been named  $\alpha_1\beta_1$  and  $\alpha_1\beta_2$ , respectively (Fig. 4-17). (The  $\alpha_2\beta_2$  contact is the same as  $\alpha_1\beta_1$ .) The  $\alpha_1\beta_1$  contact point is extensive and moves relatively little (less than 0.1 nm) when hemoglobin is oxygenated. The  $\alpha_1\beta_2$  contact is smaller and smoother, and movement on oxygenation is relatively great (as much as 0.7 nm). As a result, there are two quaternary structures for hemoglobin: one for the deoxygenated form and one for the liganded or oxygenated form. The main difference between the two is the nature of the  $\alpha_1\beta_2$  contact (Fig. 4-17).

#### Changes in Hemoglobin Structure on Oxygenation

In the deoxy state, the oxygen affinity of hemoglobin is less than that of isolated heme subunits or that of partially oxygenated

hemoglobin. The change in oxygen affinity on oxygenation is called heme-heme (or subunit) interaction and accounts for the characteristic sigmoidal shape of the hemoglobin-oxygen dissociation curve (page 107). An additional effect of oxygenation of hemoglobin is the release of protons (the alkaline Bohr effect). These changes, as well as the interaction with 2,3 DPG (page 107), have been explained on a molecular basis in a model proposed by Perutz.<sup>518</sup>

The quaternary structure of the deoxy (low oxygen affinity) form of hemoglobin is stabilized by salt bridges involving the carboxyterminal ends of the polypeptide chains. The  $\alpha$ -terminal arginine forms two salt bridges with the adjacent  $\alpha$ -chain, one with  $\alpha 1$  (NA1) valine, and another with  $\alpha 126$  (H9) aspartate. The  $\beta$ -terminal histidine forms a salt bridge with an adjacent  $\alpha$ -chain ( $\alpha 40$  (C5) lysine) as well as an "internal" salt bridge with its own  $\beta 94$  (FG1) aspartate. An additional factor stabilizing the deoxy form is 2,3 DPG (page 102), which joins the N-terminal end of one  $\beta$ -chain to the 143 (H21) histidine of the other.

The tertiary structure of deoxygenated subunits differs slightly from that of the oxygenated form. In the deoxy form, heme iron is in a high-spin state and in this form is displaced slightly from the plane of the porphyrin ring. The penultimate tyrosine is wedged firmly between the F and H helices. When oxygen is added, iron changes to a low-spin state and moves to a position in plane with the porphyrin ring, a distance of about 0.2 nm, pulling with it the F helix to which it is attached. This movement narrows the space between the F and H helices, expelling the penultimate tyrosine from its pocket. The C-terminal amino acid is carried with the tyrosine, thereby breaking the salt bridges with adjacent chains.

The sequence of molecular changes on oxygenation is as follows: the first oxygen molecule is added, probably to an  $\alpha$ -chain, with consequent displacement of the F helix, extrusion of the penultimate tyrosine, and rupture of salt bridges. Oxygen is then added to the second  $\alpha$ -chain and to the two  $\beta$ -chains

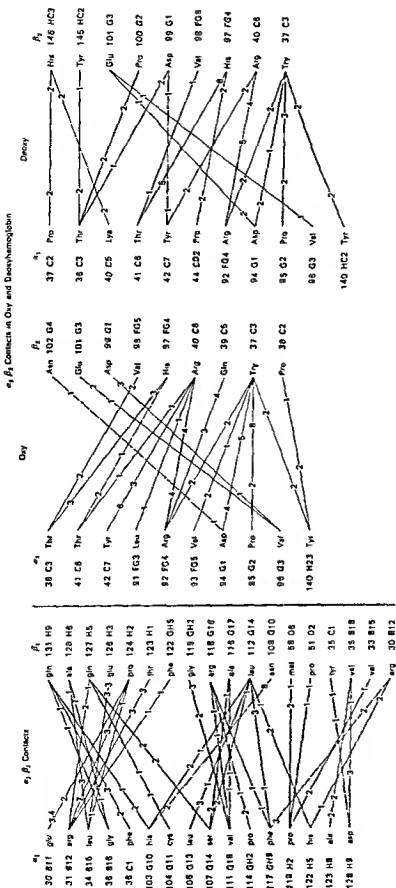


Fig. 4-17. Quaternary structure of hemoglobin. At the left is shown the most extensive contact,  $\alpha_1\beta_1$ , in which 16 amino acids in the  $\alpha$ -chain form bonds with 18 amino acids in the  $\beta$ -chain. The  $\alpha_1\beta_1$  contact does not change significantly on oxygenation. The smaller  $\alpha_1\beta_2$  contact, shown in the center and at the right, has two forms, depending on whether the hemoglobin is in the oxygenated (oxy) or deoxygenated (deoxy) form. At  $\alpha_1\beta_2$ , 10 or 11 amino acids in the  $\alpha$ -chain form bonds with nine in the  $\beta$ -chain. Plain lines indicate Van der Waals bonds and broken lines indicate hydrogen bonds. The numbers on the lines give the number of atoms in contact (From Perutz et al.,<sup>11a</sup> courtesy of the authors and Nature.)

in sequence. With each addition, the tertiary changes in each subunit occur and salt bridges are broken. At some point, probably after the second or third oxygen is added, the quaternary structure clicks to the liganded configuration, accompanied by the expulsion of 2,3 DPG and disruption of salt bridges at the  $\alpha_1\beta_2$  contact point. Oxygen affinity is now much increased and oxygen is added to the remaining  $\beta$ -chain or chains.

The alkaline Bohr effect is explained by rupture of the salt bridges involving the  $\beta$ -C-terminal histidine and the  $\alpha$ -N-terminal valine. When these bridges are broken, the  $pK$  of the dissociation of hydrogen ion is reduced. It has also been suggested that about 25% of the alkaline Bohr effect might be accounted for by histidine at  $\alpha 122$ .<sup>522</sup>

### Globin Biosynthesis and Its Genetic Control

Synthesis of globin is accomplished by the cellular protein-synthesizing mechanisms outlined in Chapter 2 (page 47). At least four pairs of structural genes are required, one for each of the four normal polypeptide chains. In addition, there is evidence that both the  $\alpha$  and  $\gamma$  structural loci are reduplicated (page 797). The hemoglobin structural genes are carried on two pairs of autosomes. The  $\alpha$ -chain structural genes occupy loci on one pair of autosomes, and the  $\beta$ -,  $\gamma$ -, and  $\delta$ -chain structural genes occupy loci close to one another on a second pair of autosomes (page 797). A "point mutation" in one of these structural genes leads to the production of an abnormal hemoglobin characterized by the substitution of a single amino acid for another. Such abnormal hemoglobins are discussed further in Chapter 24.

In addition to the structural genes, regulator genes that control the rates of synthesis of various polypeptides are thought to exist.<sup>510,513,525</sup> Such genes may account for the changes from  $\epsilon$ - to  $\gamma$ - and from  $\gamma$ - to  $\beta$ -chain production as the fetus and infant mature. Theoretically, a mutation at one of these controller gene loci can result in

changes in the rate of synthesis of a polypeptide chain without any alteration in its structure. Such a mutation might lead to thalassemia or to the hereditary persistence of hemoglobin F (Chapter 26).

### Synchronization of Heme and Globin Synthesis

Within the erythrocyte precursor cell, heme and globin are made in nearly equivalent amounts. If heme biosynthesis is restricted, eg, in iron deficiency, a comparable reduction in globin synthesis occurs.<sup>512</sup> Furthermore, addition of heme to iron-deficient reticulocyte preparations stimulates globin synthesis.<sup>503</sup> This synchrony between the rates of globin and heme synthesis appears to be accomplished by an effect of heme on globin synthesis at the translational level.<sup>520</sup> In the absence of heme, the polyribosomes disaggregate and globin synthesis ceases. The disaggregation is reversible, and, if heme is added, the ribosomes again form into polyribosomes and globin synthesis begins. It is likely that heme acts at the initiation stage of polypeptide chain synthesis. Both positive and negative effects of heme have been observed. A repressor of chain initiation that is inactivated by heme has been described.<sup>520</sup> In addition, a factor promoting chain initiation may be activated by combining with heme.<sup>511</sup>

### Evolution of Hemoglobin<sup>502,514</sup>

The relatively complicated, tetrameric, and chemically highly organized nature of the hemoglobin molecule and the evidence for genetic control of its primary sequence have led to the development of a phylogenetic theory of hemoglobin structure. The primary structures of the  $\alpha$ - and  $\beta$ -chains are very similar. Fifty-nine of the amino acids are identical in the two chains in the order presented in Table 4-7. With only minor shifting of gaps to maximize homology, 64 amino acids may be considered identical.<sup>502</sup> Still greater homology occurs between the  $\beta$ -,  $\gamma$ -, and  $\delta$ -chains. It is postulated that the hemoglobin molecule originally was much simpler

than at present, initially consisting of a single peptide chain, probably very similar to myoglobin. To explain the appearance of new peptide chains it has been proposed that a gene duplication took place, each gene corresponding to one of the two peptide chains, which were originally identical. From this time on, the genetic material developed independently and underwent different mutations. By gene duplication,  $\gamma$ -,  $\beta$ -, and  $\delta$ -chains were produced. Dimers and tetramers of the peptide chains, such as  $\alpha\beta$  and  $\alpha_2\beta_2$ , were formed. The appearance of subunit interaction brought with it a marked increase in the physiologic effectiveness of the molecule. Such, it is postulated, are the non-lethal mutations in the hemoglobin molecule that have taken place in the past 500,000 years. On the basis of an analysis of discontinuities ("sequence gaps") in the homologous architecture of the peptide chains, attempts have been made to postulate the constitution of the original hemoglobin molecule. Braunitzer believes that the peptide chain of the hemoglobin ancestor was longer than the present  $\alpha$ - and  $\beta$ -chains. There also is reason to expect that a phylogeny based solely on molecular data derived from the study of the hemoglobins of various animal species can ultimately be developed.<sup>505</sup>

## Control of Erythropoiesis<sup>505</sup>

It is evident that a well-balanced mechanism exists that maintains the erythron within "normal" limits and mediates the response to a variety of normal and abnormal situations. In broad outlines, this control system operates in the following manner. Alterations in the concentration of hemoglobin in the blood lead to changes in tissue oxygen tension within the kidney. In response to hypoxia, the kidney secretes a factor that interacts with a plasma substrate to produce a hormone, erythropoietin. This hormone induces primitive marrow cells to differentiate into pronormoblasts, thereby bringing about expansion of the erythroid marrow and an increase in red cell production. This, in turn, leads to an increase in the size of the

erythron and an increase in tissue oxygen levels. Each of the major steps in this process will be discussed in greater detail in the sections to follow.

## Tissue Oxygen

Tissue oxygen tension depends on the relative rates of oxygen supply and demand. Oxygen supply is a complex function of interacting, but semi-independent variables, including (1) blood flow, (2) blood hemoglobin concentration, (3) hemoglobin oxygen saturation, and (4) hemoglobin oxygen affinity. Each of these functions may be altered to compensate for a deficiency in one of the others. For example, in severe anemia, cardiac output and respiratory rate may increase (page 534) and hemoglobin oxygen affinity may be reduced through the 2,3 DPG effect (page 107). Conversely, in respiratory insufficiency, secondary polycythemia occurs.

Despite the influence of cardiovascular and respiratory adjustments, tissue oxygen tension decreases roughly in proportion to the degree of anemia.<sup>560,632</sup> Conversely, induced polycythemia of moderate degree leads to normal or increased tissue oxygen tension and to increased tolerance to hypoxia.<sup>614,632,636</sup> These changes occur despite the increase in blood viscosity that accompanies polycythemia (page 124), suggesting that peripheral vascular resistance decreases to compensate for increased viscosity. However, with advanced degrees of polycythemia, the increase in viscosity may be great enough to negate the advantages of increased oxygen-carrying capacity.

That tissue hypoxia is the fundamental stimulus to erythropoiesis was first suggested by Miescher in 1893.<sup>613</sup> This concept has been amply confirmed.<sup>566,587</sup> However, it now seems clear that hypoxia does not exert its effects by a direct action on the marrow,<sup>612,631</sup> as Miescher believed, but, instead, acts by inducing the elaboration of a hormone, erythropoietin. The nature of the tissue oxygen receptors remains unknown; however, these receptors probably are located within the kidney since production of eryth-

ropoietin can be induced by renal artery constriction<sup>577</sup> or by hypoxic perfusion of the isolated kidney.<sup>576</sup>

**Erythropoietin<sup>560,575,583,584,587,591,595</sup>  
(Erythropoiesis Stimulating  
Factor, ESF)**

In 1906, Carnot and Déflandre proposed that the circulating blood carries an erythropoietic stimulating substance, "hémopoétine."<sup>559</sup> Their work was ignored, in part because they provided scanty experimental data to support their view. The report in 1950 that normoblastic hyperplasia of the bone marrow developed in both partners of parabiotic pairs of rats, only one of which was subjected to hypoxia,<sup>620</sup> renewed interest in the possibility that the hypoxic stimulus is mediated through a hormone-like factor in the blood. This possibility found additional support when a generalized increase in erythropoiesis was observed in a patient with localized hypoxia due to an A-V fistula.<sup>631</sup> Subsequently it was shown that nursing mice, kept at low oxygen tension except when suckling their young, secrete in the milk a substance that causes polycythemia in the nurslings<sup>587</sup>; furthermore, when larger amounts of plasma from anemic animals than were used in Carnot's studies were injected intravenously into normal recipients, a more impressive reticulocytosis occurred than had been observed before, and the quantity of circulating hemoglobin increased as well.<sup>566,583</sup>

It is now generally accepted that these observations can be accounted for by the effects of a hormone, erythropoietin, the production of which is related to tissue oxygen tension.

**Chemical Properties and Bioassay**

Highly purified erythropoietin has been prepared from the plasma of sheep made anemic with phenylhydrazine.<sup>581</sup> The specific activity of this preparation was 8250 units per mg of protein, indicating a more than million-fold purification, and the only contaminant

appeared to be erythropoietin lacking its sialic acid moiety. A similar degree of purity (8300 units/mg) was achieved in material prepared from the urine of anemic human subjects with hookworm disease.<sup>572</sup>

Erythropoietin is relatively stable to heat and storage. It is a glycoprotein containing sialic acid, hexoseamine, and hexoses. If the sialic acid is removed, the erythropoietic activity is lost *in vivo*,<sup>625</sup> but is retained *in vitro*. Estimates of molecular weight have ranged from 27,000 to 100,000.<sup>575,584</sup> With highly purified sheep plasma erythropoietin, molecular weight was reported to be 45,800, of which 30% was carbohydrate.<sup>581</sup> Erythropoietin migrates as an  $\alpha$ -globulin during electrophoresis.

Erythropoietin usually is detected and quantitated by means of bioassay. The most sensitive and economical assays employ ex-hypoxic, polycythemic (erythropoietically depressed) mice as the test animal and the incorporation of <sup>59</sup>Fe into circulating erythrocytes as the index of erythropoietic activity.<sup>575,584</sup> Immunologic assays, including a hemagglutination-inhibition technique and a radioimmunoassay,<sup>579</sup> appear promising but are still in various stages of development. Erythropoietin activity is expressed in units, and 1 unit is defined as that amount of activity in 1.48 mg of the first international reference preparation (IRP) of erythropoietin, formally called Erythropoietin Standard B,<sup>562</sup> or in 0.5 mg of the second IRP.<sup>555</sup> These units are approximately equivalent to the older cobalt unit, which was defined as the erythropoietic activity produced by the administration of 5  $\mu$ moles of cobalt chloride. The IRP's were prepared from pooled urine of anemic human subjects and standardized by bioassay in a number of independent laboratories. They are made available by the National Institute for Medical Research, London, England.

**Secretion**

The major site of erythropoietin production is the kidney.<sup>583,590,626,630</sup> In the dog the kidney is the exclusive source of the hor-

none.<sup>584,610</sup> In most other species, including man, removal of the kidneys does not abolish erythropoiesis nor bring about the complete disappearance of erythropoietin activity from plasma.<sup>570,580,584,607,608</sup> Furthermore, hypoxia stimulates the production of erythropoietin in renoprival subjects. Thus, in these species there are non-renal, erythropoietin-producing tissues. The liver appears to be one such tissue,<sup>580</sup> but there may be others. It has not been established that the renal and extrarenal hormones are chemically identical.

It has been difficult to extract erythropoietin from normal kidneys. However, Gordon and associates have shown that incubation of renal extracts with normal plasma leads to production of an erythropoietically active substance.<sup>561,584,585,637</sup> On the basis of their observations, they proposed that the kidney makes an enzyme called *erythrogenin* (renal erythropoietic factor, REF) that is erythropoietically inactive by itself but that reacts with a plasma substrate, *erythropoietinogen*, of hepatic origin to produce active erythropoietin. These studies have been confirmed in some,<sup>599,639</sup> but not all,<sup>569</sup> laboratories. An alternative proposal holds that erythropoietin is present in the kidney, but that its activity is inhibited, possibly by a lipid substance.<sup>573</sup> According to this proposal, plasma incubation acts by destroying or removing the inhibitor. However, the renal secretion of erythropoietin in response to a hypoxic stimulus requires DNA-dependent RNA production and the de novo, ribosomal synthesis of protein.<sup>627</sup> Therefore, it cannot be accounted for by release of preformed, inhibited hormone.

The location of the erythropoietin-secreting mechanism within the renal parenchyma remains controversial. One body of information supports the juxtaglomerular apparatus (JGA) as the secretory organ. In some experimental situations associated with increased erythropoiesis, hyperplasia and increased granularity of the JGA have been observed. Furthermore, erythropoietin release has been associated with discharge of granules from the JGA,<sup>564</sup> and a patient has been described with polycythemia and in-

creased erythropoietin production with histologically proved JGA hypertrophy (Bartter's syndrome).<sup>593</sup> However, many of these observations may relate to the known role of the JGA in renin secretion rather than erythropoietin production.<sup>584</sup> In studies employing the fluorescent antibody-staining technique, erythropoietin was found to be localized in the epithelial cells of the glomerulus, and no staining of the JGA was observed.<sup>578</sup> It was suggested that the glomerulus is the site at which *erythrogenin* reacts with its substrate. Additional support for a glomerular site of erythropoietin synthesis is the observation that tissue cultures derived from isolated goat kidney glomeruli produced erythropoietin over periods of seven months.<sup>557</sup>

Unlike erythropoietin, erythrogenin appears to be formed diffusely throughout the kidney.<sup>584,629</sup> Medullary, cortical, and isolated glomerular or tubular fractions all are active. In subcellular preparations, erythrogenin activity was found in "light mitochondrial" and "microsomal" fractions. On the basis of the time course of hypoxia-induced changes in these fractions, it was proposed that erythrogenin is secreted in the endoplasmic reticulum of microsomes and stored in the peroxisomes of the "light mitochondrial" fraction.<sup>558</sup>

## Action

The primary site of action of erythropoietin appears to be an unidentified stem cell (the "erythropoietin sensitive cell,"<sup>584</sup> page 51). This cell is conceived to be committed to the erythrocyte cell line and a precursor to the pronormoblast. Addition of erythropoietin to *in vitro* cultures of rat bone marrow leads to an increase in iron uptake and the synthesis of a species of RNA with a sedimentation coefficient of 9s.<sup>592</sup> Presumably, the latter is the messenger for globin synthesis. Thus, erythropoietin appears to activate controller and structural genes essential to hemoglobin synthesis, and in so doing causes the erythropoietin-sensitive cell to assume the morphologic features of a pronormoblast. In addition, there is evidence

that self-replication and proliferation of the erythropoietin-sensitive cells are stimulated by erythropoietin.<sup>621</sup> Ultimately, these effects lead to expansion of the marrow erythrocyte production compartment (erythroid hyperplasia).<sup>615</sup>

Erythropoietin also may have other actions.<sup>584, 615</sup> Red cell generation time is shortened, cellular divisions may be skipped,<sup>628</sup> premature denucleation occurs, and reticulocytes are released to the blood at an earlier stage of maturity.<sup>615, 618</sup> These mechanisms result in the release of a population of erythrocytes that are macrocytic, hypochromic, and polychromatophilic and have a relatively short life span. Obviously, such a population has only limited utility and can contribute to improved function for only a short time.

#### Erythropoietin Levels in Health and Disease

Erythropoietin can be detected in both urine and plasma. In various laboratories employing the polycythemic mouse assay, normal values for urine erythropoietin in men have been reported to be 1 unit/day,<sup>634</sup>  $2.8 \pm 1.3$  units/day (mean  $\pm$  1 SD),<sup>552</sup> or  $4.2 \pm 1.3$  units/day.<sup>549</sup> Values in women were about 50% lower. It seems likely that the rate of urinary excretion bears a relation to the rate of production of the hormone. However, the amount in the urine constitutes less than 10% of the amount produced, and little is known regarding the fate of the remainder.<sup>554</sup>

With the mouse assay, erythropoietin has also been detected in normal plasma,<sup>606</sup> but the level cannot be measured with accuracy until it reaches about three times normal. With an immunologic assay, normal values of 7.5 to 60 mU per ml of plasma were reported.<sup>597</sup>

The occurrence of erythropoietin in the urine and plasma of normal persons constitutes evidence that the hormone is required for normal erythropoiesis. Additional support for this belief comes from the observations

that administration of antibodies to erythropoietin depresses normal erythropoiesis,<sup>598, 624</sup> and that hypertransfusion of normal subjects decreases urinary erythropoietin levels.<sup>549</sup>

When anemia is induced in normal persons by phlebotomy, urinary erythropoietin levels increase, and an inverse relationship between the VPRC and the logarithm of urinary erythropoietin can be demonstrated<sup>549</sup> (Fig. 4-18). A similar relation probably exists in a variety of situations in which an intact erythropoietin-secreting system is associated with anemia, including iron-deficiency anemia, pernicious anemia, leukemia, aplastic anemia, sickle cell anemia, and others.<sup>609, 634, 635</sup> There are, however, several conditions in which the erythropoietin level in urine or plasma is lower than would be predicted by the curve in Figure 4-18. These include the anemias of renal insufficiency,<sup>551</sup> chronic disorders (cancer, infection, and rheumatoid arthritis)<sup>633</sup> (Chapter 18), protein-calorie malnutrition (page 136), certain endocrine deficiency states (Chapter 19), and the anemia associated with abnormal hemoglobins with reduced oxygen affinity, such as hemoglobin Seattle (page 815). The low levels in these disorders suggest that either reduced erythropoietin secretion is a factor in the pathogenesis of the anemia or tissue oxygen delivery is appropriate to body needs despite reduced hemoglobin concentration.

Erythropoietin levels also have been studied in conditions associated with polycythemia. A prompt increase in plasma erythropoietin occurs in individuals subjected to acute hypoxemia,<sup>549, 573</sup> and increased values are found in permanent residents of high altitudes.<sup>631</sup> In patients with polycythemia secondary to hypoxia, erythropoietin levels are usually increased, sometimes greatly so, but if the polycythemia adequately compensates for the hypoxia, erythropoietin may return to normal limits.<sup>549</sup> On the other hand, in polycythemia vera, erythropoietin levels are uniformly low, and red cell production in this illness appears to be independent of the usual control system.<sup>549</sup> Finally, a number of instances of polycythemia associated

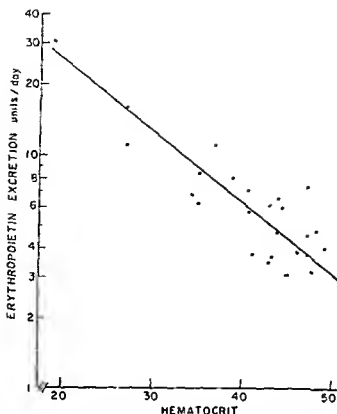


Fig 4-18. Relation of the volume of packed red cells (hematocrit) to urine erythropoietin (log scale) in normal individuals subjected to phlebotomy. The same relationship probably holds for many common anemias in which the erythropoietin secreting mechanism is intact. If points fall significantly below the line, this suggests that defective erythropoietin secretion may be important in the pathogenesis of the anemia. (From Adamson,<sup>549</sup> courtesy of the author and Henry M. Stratton, Inc.)

with tumors, especially hypernephromas, cerebellar hemangioblastomas, and hepatomas, have been reported (page 986). In some of these, erythropoietin-like materials were found in urine or plasma or in extracts of the tumor, suggesting that this association represents unregulated secretion of erythropoietin by neoplastic tissue.<sup>549</sup>

### Role of the Nervous System

Regulation of erythropoiesis by the nervous system has long been postulated, but the evidence has been unconvincing.<sup>557</sup> More recently, electrical stimulation of brain areas in or near the hypothalamus has induced reticulocytosis, increases in the red cell mass, and increases in erythropoietin production.<sup>574,558,601</sup> Also, the induction of large,

hypothalamic lesions has inhibited the erythropoietic response to hypoxia.<sup>589,604</sup> Finally, atropine has been shown to prevent the reticulocytosis of hypothalamic stimulation as well as the erythropoietin response to hypoxia.<sup>575</sup>

The physiologic importance of these observations remains to be established. It is possible that the hypothalamus senses tissue oxygen levels and stimulates erythropoiesis, either through a neurohypophyseal humoral mechanism or via the sympathetic nervous system. Alternatively, the observed effects may result from unphysiologic variations in vascular dynamics or blood oxygenation.<sup>544</sup> Since the deoxygenated human renal homograft can resume erythropoietin production,<sup>563</sup> it is unlikely that this function is mediated by nerve fibers.



### Other Erythropoietic Substances

The importance of *androgens* in erythropoiesis has long been inferred from the observed differences between adult men and women in the measures of red cell concentration. Values for the volume of packed red cells, blood hemoglobin concentration, and red cell count in males exceed those in females, the differences becoming apparent at puberty (see Appendix, Table A-1). Castration in adult males is followed by a reduction in the above measures to levels typical of females. Furthermore, administration of physiologic doses of androgens to patients with hypogonadism or panhypopituitarism leads to an increase in the volume of packed red cells.<sup>563,592,593</sup> Finally, in pharmacologic doses, androgens may bring about improvement in certain, otherwise refractory anemias, such as aplastic anemia (Chapter 56), myelofibrosis (Chapter 57), or sideroblastic anemia (Chapter 18).

There appear to be several mechanisms whereby androgens exert their effects. There is little doubt that they stimulate erythropoietin production.<sup>553,599,622</sup> This effect may be partially explained by the induction of renal hypertrophy,<sup>600,601</sup> but there is an additional, more rapid action since increased erythropoietin secretion can be demonstrated in isolated kidneys perfused with androgens.<sup>599</sup> Androgens may also potentiate the action of erythropoietin, perhaps by increasing the number of erythropoietin-sensitive stem cells.<sup>602,611</sup> Finally, there are studies suggesting that androgens exert a direct effect on erythropoiesis without the intermediation of the erythropoietin system.<sup>567,589,619</sup>

*Estrogens* may exert an inhibitory effect on red cell production. Castration in female rats causes an increase in the red cell count, and administration of estradiol is followed by a drop in the elevated counts. In small doses, estrogens may decrease the stem cell response to erythropoietin.<sup>592</sup> In very large doses, they may suppress erythropoietin production.<sup>605</sup>

A mild to moderate anemia is observed with deficiency of *thyroid hormone* in myxedema or following thyroidectomy or pro-

longed administration of antithyroid drugs (Chapter 19). The anemia may be corrected by administration of thyroid hormone. It is likely that thyroid hormone exerts its effects on erythropoiesis indirectly, by altering the tissue demand for oxygen. Similarly, *cortisol*<sup>592</sup> and *growth hormone*<sup>623</sup> probably also affect erythropoiesis secondarily by a similar mechanism. Evidence for the oxygen demand hypothesis is considered in Chapter 19 (page 711).

Certain other hormones may affect red cell production. *Placental lactogen*<sup>592</sup> and *prolactin*<sup>592</sup> appear to stimulate erythropoiesis directly, but erythropoietin must be present for maximum effect. A number of *vasoactive hormones* stimulate erythropoiesis, probably by decreasing blood flow to the renal hypoxia-sensing mechanism, thereby inducing erythropoietin secretion.<sup>575</sup> These vasoactive materials include vasopressin, angiotensin, 5-hydroxytryptamine, norepinephrine, and prostaglandin E.

The *carotid body* has been implicated as a source of an erythropoietic hormone in cats.<sup>633</sup> Other investigators, however, found, instead, that the erythropoietic response of cats to hypoxia was enhanced by carotid body removal.<sup>617</sup> Furthermore, no evidence of impaired erythropoiesis could be detected in 57 human subjects who had had both carotid bodies removed.<sup>598</sup>

*Cyclic adenosine monophosphate (cAMP)* has been shown to stimulate erythropoiesis in rodents.<sup>536,626</sup> Since this effect was blocked by an erythropoietin antibody, it was considered likely that cAMP acted by stimulating erythropoietin production.<sup>626</sup> In addition, however, a direct effect on the marrow was suggested by the observation that cAMP induced the activity of aminolevulinic acid synthetase (page 169) in rabbit bone marrow cultures.<sup>556</sup> There was, however, no increase in iron uptake or in overall heme synthesis in such a system.<sup>586</sup>

The injection of *hemolysates*, *hemoglobin*, or *hemin* has been shown to stimulate erythropoiesis, and this phenomenon has been offered as an explanation for the increased erythropoiesis in patients with compensated

hemolytic anemia and normal blood hemoglobin levels. The effect of these materials appears to depend on erythropoietin secretion<sup>571,613</sup> and may well represent a toxic or pharmacologic effect on the kidney rather than a physiologic mechanism.<sup>571</sup>

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## *Destruction of Erythrocytes*

The Life Span of the Erythrocyte  
Erythrocyte Aging  
Mechanisms of Red Cell Destruction  
Sites of Erythrocyte Destruction  
Hemoglobin Catabolism

IN the preceding chapters the structure, chemical composition, and metabolism of the red cell were discussed, as were the substances required for hemoglobin production and the manner in which hemoglobin synthesis is accomplished. The red corpuscle is a highly specialized structure that loads, transports, and unloads oxygen with speed and efficiency and yet consumes little of this vital gas itself. Its survival depends upon its ability to maintain its unique physical properties despite the chemical variations in its environment and the continuous physical abuse to which it is subjected in the circulation. The biologic truism that, as specialization increases, adaptability and durability are more or less proportionately diminished applies to the red corpuscle. Being incapable of much repair, it is ultimately destroyed. The continuous excretion of bile pigment gives evidence of the continuity of the process of red cell breakdown.

### **The Life Span of the Erythrocyte**

Valid measurements of erythrocyte survival in the intact animal became possible in 1919 when Ashby devised the differential agglutination technique.<sup>2</sup> Since then, other, less cumbersome methods have been intro-

duced. Studies with these techniques have demonstrated that, after a finite life span, the erythrocyte becomes nonviable and disappears from the circulation. In man, this life span averages approximately 120 days. In other mammals, values range from as short as 40 days in mice to as long as 225 days in the llama.<sup>3</sup> Still longer survivals, 600 to 1400 days, are observed in poikilothermic reptiles with low metabolic rates, such as toads and turtles.<sup>1</sup>

In addition to red cell destruction associated with senescence, there is some degree of random erythrocyte breakdown. The latter is minimal in normal man, but may increase greatly under pathologic circumstances. In other species, especially the pig<sup>5</sup> and the llama,<sup>19</sup> a greater degree of random destruction than in man is observed under normal circumstances.

### **Methods for Estimating Erythrocyte Life Span**

Erythrocyte life span may be determined indirectly from ferrokinetic studies (page 164) or from measurements of the rate of heme catabolism (page 216). There are also a number of direct methods for measuring erythrocyte life span. These fall into one of two categories.<sup>3,4</sup> Cohort methods depend on the incorporation of an isotopically labeled substance into a group ("cohort") of newly formed cells. If exposure to the label is brief and if there is no reutilization of label, the tagged cells will be of very nearly the same age. In contrast, random-labeling methods utilize tracers that bind with all cells in the circulation regardless of age. The patterns of

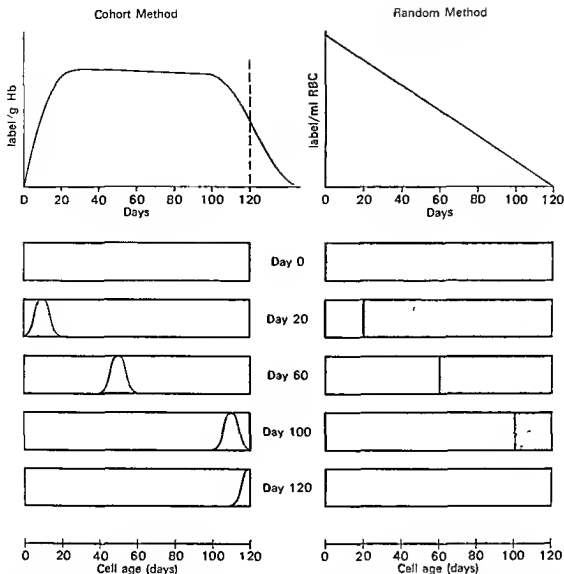


Fig. 5-1 Comparison of idealized patterns produced by cohort and random methods of labeling circulating erythrocytes. These curves are constructed on the assumption that there is no elution of label and that all erythrocyte destruction is senescent. In the bar below the main graphs the distribution of labeled cells (shaded areas) by cell age is indicated. These labeled cells can be thought of as being forced to the right by a piston made of newly produced, unlabeled cells (Modified from Berlin,<sup>4</sup> courtesy of the author and JAMA)

time-dependent change in circulating red cell label produced by these two procedures are quite different (Fig. 5-1). Cohort labels result in a curve characterized by a plateau, the length of which is a measure of erythrocyte life span. In contrast, random labels begin to disappear from the circulation immediately and erythrocyte life span is related to the time when all the label has vanished, the so-called extinction time.

### Cohort Methods

Precursors used for cohort labeling include  $^{59}\text{Fe}$ ,  $^{75}\text{Se}$ -methionine,<sup>24</sup> and glycine tagged with  $^{15}\text{N}$ ,  $^{14}\text{C}$ , or  $^3\text{H}$ . These are incorporated into hemoglobin synthesized by the erythrocyte precursors and remain with the cell throughout its life span. Another cohort-labeling method<sup>6</sup> employs a combination of non-radioactive diisopropylfluorophosphate

(DFP) and  $DF^{32}P$ . In order to determine erythrocyte life span with cohort labels, assays must be performed for 135 to 150 days if the red cell life span is normal. Of the available cohort labels,  $^{59}Fe$ ,  $^{75}Se$ -methionine, and  $DF^{32}P$  are reasonably safe and relatively easy to assay. The requirement for the use of a mass spectrometer limits the usefulness of  $^{15}N$ -glycine,  $^3H$ -glycine and  $^{14}C$ -glycine, which emit low-energy beta particles, must be assayed by liquid scintillation or low background Geiger counting. Both isotopes have dangerously long physical and biologic half-lives.

### Random-Labeling Methods

Random-labeling methods have proved to be much more useful than cohort methods for both clinical and research applications because accurate information is made available in a relatively short time. Once enough assays have been made to establish the form of the disappearance curve, it may be extrapolated to the base line to determine the extinction time. The latter is directly related to the finite (senescent) life span. More frequently, the time at which half of the label has disappeared from the circulation ( $t_{1/2}$ ) is recorded. When there is neither a significant degree of random destruction nor elution of label, the form of the disappearance curve is linear, and mean erythrocyte life span is equal to twice the value for  $t_{1/2}$ .

Random methods include those based on the Ashby differential agglutination technique as well as those in which cells are labeled with  $^{51}Cr$ ,  $^3H$ -DFP or  $DF^{32}P$ . Preliminary studies indicate that  $^{14}C$ -cyanate may also be a useful, non-eluting random label.<sup>20</sup> The Ashby method<sup>2,9</sup> requires the transfusion of compatible but immunologically identifiable blood, eg, group O cells into a group A recipient. At appropriate intervals, the donor red cells are enumerated following agglutination or hemolysis<sup>17</sup> of the recipient's cells by appropriate antisera. Methods for automating the procedure have been described.<sup>23</sup> When properly performed the differential agglutination technique yields ac-

curate results. The observed disappearance curves are linear in normal human subjects, and deviation from linearity indicates random destruction. Inability to measure the survival of a subject's cells in his own circulation as well as the hazards of transfusion and the exacting requirements of technique limit the usefulness of the method.

Radioactive chromium was introduced as a red cell label in 1950<sup>14</sup> and was utilized in survival studies in vivo shortly thereafter.<sup>10,11,22</sup> Anionic (hexavalent) chromium in the form of the chromate ion ( $^{51}CrO_4^{2-}$ ) can penetrate the red cell membrane. Once inside the cell it is converted to the trivalent cation ( $Cr^{+3}$ ) and in this form becomes firmly, but not irreversibly, bound to hemoglobin. Cationic chromium may be used to tag plasma proteins, but it will not penetrate, and therefore will not label, the erythrocyte. Because of the change to the cationic form, there is no reutilization of the chromium label. The chromium attaches to the beta chains of hemoglobin A and the gamma chains of hemoglobin F.<sup>15,23</sup>

The chromium method is probably used more than any of the other available methods. Several factors account for its popularity. Since it emits a high-energy gamma ray, radioassay is comparatively easy and requires little sample preparation. Furthermore, radioactivity can be monitored over the body surface to determine the principal sites of destruction. Since labeling can be, and usually is, performed ex vivo, cross-transfusion studies are possible; thus, survival of a patient's cells in a normal recipient or of normal cells in a patient can be evaluated. Still another advantage of the ex vivo chromium method is that red cell volume also may be determined (page 122).

The principal disadvantage of the chromium label is that it is slowly eluted from the cell. The rate of elution in normal subjects was found to be 0.57 to 1.28% per day and averaged 0.93% per day.<sup>10,11</sup> In most patients with various hematologic diseases, the chromium elution rate was 0.62 to 2.27% and averaged 1.29% per day; in a small number a second, much more rapid, elution rate

constant was observed, namely 8 to 14% per day.<sup>7</sup> An assumed rate of 1% per day is often used for analysis of chromium survival curves (see below). The disappearance of  $^{51}\text{Cr}$  from the circulation thus reflects not only the red cell life span, but also the elution rate. Another consequence of chromium elution is a nonlinear disappearance curve. However, if the data are plotted on a semilogarithmic scale, or if appropriate correction factors<sup>18</sup> are applied (Fig. 5-2), the pattern approximates a straight line. From such a plot, the disappearance half-time ( $t_{1/2}\text{Cr}$ ) may be derived.

The chromium elution rate depends in part on the technique used in the labeling procedure.<sup>21</sup> Consequently, reported average normal values for  $t_{1/2}\text{Cr}$  have varied widely, from 25 to 33 days. In order to standardize methodology, an international committee has specified a labeling procedure and, furthermore, has recommended that the use of  $t_{1/2}\text{Cr}$  as a method of expressing results be abandoned.<sup>18</sup> Instead, a mean red cell life span is to be calculated. To do so, the chromium survival data should first be corrected for elution by means of correction factors specified by the committee. Then the data should be made to fit either an arithmetic (linear) or an exponential disappearance curve by means

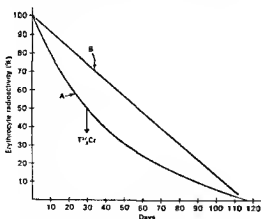


Fig 5-2  $^{51}\text{Cr}$  erythrocyte survival curve in a normal subject before (A) and after (B) correction for elution (see text). Half of the radioactivity is gone at 30 days ( $t_{1/2}\text{Cr}$ ) but the radioactivity does not disappear completely until 115 days, the so-called extinction time

of a least-squares fitting procedure. The "goodness of fit" is assessed. If the data best fit a linear plot, the mean red cell life span is determined from the reciprocal of the slope, eg, by multiplying the  $t_{1/2}$  by 2. If they best fit a semilogarithmic plot, the reciprocal is obtained by multiplying the  $t_{1/2}$  by 1.44. In theory, a linear disappearance curve should be found with senescent destruction and an exponential curve with random destruction. A possible objection to the recommended method of analysis is that the correction factors assume a single value for the  $^{51}\text{Cr}$  elution rate, even though variation within the normal and in disease is to be expected. For this reason, until the committee's recommendations have been fully accepted, both a  $t_{1/2}\text{Cr}$  and a mean red cell life span had best be calculated.

Diisopropylfluorophosphate labeled with  $^{32}\text{P}$  ( $\text{DF}^{32}\text{P}$ ) reacts with esterases, especially cholinesterase, and becomes firmly bound to the cell. In contrast to chromium, DFP is not eluted after the first 24 to 48 hours. As a result, the normal disappearance curve is linear and many complexities of interpretation are avoided.<sup>12,13</sup> For these reasons,  $\text{DF}^{32}\text{P}$  is probably the most satisfactory label for determining life span, although sample preparation is somewhat complicated because the usually available equipment for detecting the high-energy beta particles emitted by  $^{32}\text{P}$  requires that the samples be taken to dryness. Labeling is most effectively accomplished in vivo; a single injection of  $\text{DF}^{32}\text{P}$ , not to exceed 20  $\mu\text{g}/\text{kg}$  body weight, in propylene glycol is administered intravenously over a period of 10 to 15 minutes.<sup>6,18</sup> Ex vivo labeling is possible, but a large volume of blood (100 to 200 ml) is required<sup>16</sup> and cross-transfusion studies are therefore cumbersome. The isotope cannot be used if counting over body surfaces is desired.

### Double Labeling

Double labeling makes possible the comparison of two erythrocyte populations, eg, recipient and donor populations, in a single

host, and is one means of distinguishing intracorporeal and extracorporeal hemolytic mechanisms (page 720). Double labeling may be accomplished with the use of immunologic and isotopic methods<sup>10</sup> or with two isotopes.<sup>8,10</sup>

### Comparison of Cohort- and Random-Labeling Methods<sup>3</sup>

Some of the advantages and disadvantages of the cohort- and random-labeling procedures have been discussed in the preceding paragraphs. The random-labeling methods provide the more rapid means for determining the red cell life span, but it may be difficult to distinguish between a shortened but finite life span and random destruction

of low intensity, if this distinction is important.

It is usually desirable to perform studies of erythrocyte life span in the steady state, i.e., when erythrocyte production and destruction are equal and the circulating red cell volume remains constant over the period of study. If these conditions are not met, certain errors in interpretation are to be expected. These errors generally are greater with random-labeling methods than with the cohort-labeling techniques. For example, if it is assumed that the red cell volume is constant when, in fact, it is decreasing, then the rate of change of label per ml RBC will be factitiously low, and the red cell life span will be estimated as being longer than it really is (D in Fig. 5-3). In the extreme, if production of red cells is at a standstill, no change in

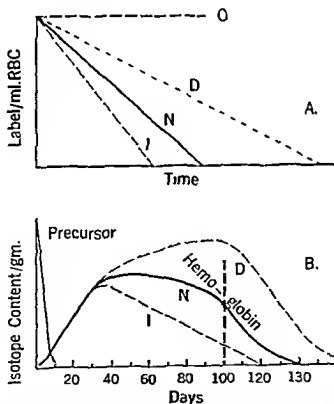


Fig 5-3. Schematic diagrams of data derived from random labeling of red cells (A) and from cohort labeling (B). N indicates the normal findings. The effect of a changing total red cell volume on the measurement of red cell life span is also shown. O indicates absence of erythropoiesis. D refers to decreasing red cell volume. I refers to increased red cell volume. (Modified from Berlin<sup>3</sup>)



label content per ml RBC would be detected and the red cell life span would appear to be infinite (O in Fig. 5-3). When the rate of production increases and the total red cell volume is increasing, the calculated red cell life span would be falsely short (I) as compared with the normal (N).

External bleeding results in an apparent shortening of red cell life span. If a random-labeling method is used and 20 ml of red cells are lost per day by any route, the red cell life span will appear to be only half normal. The only completely satisfactory solution for problems arising from changes in total circulating red cell volume during the course of life span measurements consists in determining the changes in total circulating isotope by measurement of the total red cell volume. A partial correction can be achieved by expressing the data in terms of radioactivity per ml of whole blood. A changing red cell mass also leads to factitious changes in the plateau-type curve derived from cohort labeling (Fig. 5-3, B).

The life span of the normal erythrocyte as measured by the various procedures described above is essentially the same, namely, 117 (110 to 135) days by the Ashby method, 113 (108 to 120) days when the  $^{51}\text{Cr}$  label was measured to extinction, 118 (109 to 127) days with  $^{15}\text{N}$ -glycine, and 124 days with  $\text{DF}^{32}\text{P}$ . Thus it appears that approximately 0.83% of the circulating red cells is replaced each day.

### Clinical Application of Life-Span Measurements

Findings in various pathologic states will be discussed in later chapters. In spite of their limitations, measurements of red cell life span have been found useful in the study of unexplained anemias not clarified by simpler means. The life span of red cells may be shortened as the result of some intrinsic defect such as occurs in the hemoglobinopathies and in red cell enzyme deficiencies or because of some extrinsic mechanism that causes the cell to be removed prematurely, as in the presence of hemolytic antibodies. These can

be distinguished by cross-transfusion studies utilizing a normal subject as donor and/or recipient. The type of curve of disappearance of red cells also can be of value. Thus, when the results are plotted on arithmetic graph paper, the course of elimination of red cells from the patient's circulation may be slow, uniform, and practically straight, indicating a senescent form of destruction. In contrast, in the acquired hemolytic anemias the rate of elimination is not only accelerated but it is at first rapid and then gradually slows (exponential). This indicates random destruction of red corpuscles without relation to their age.

In many instances, however, life-span measurements only confirm, and express in quantitative terms, what has been suspected already. Unfortunately, their importance has sometimes been exaggerated. Not only must technical limitations be given full consideration, but the role of multiple biologic influences must receive due attention.

## Erythrocyte Aging

Once the erythrocyte has lost its nucleus, mitochondria, and ribosomes, it no longer has the ability to synthesize proteins. During its subsequent existence, certain of the red cell enzymes gradually lose activity with a consequent deterioration of the metabolic processes dependent upon them (Table 5-1). Because such changes occur in the glycolytic pathway (Table 5-1), ATP becomes less available as the cell ages. Because ATP is the major source of energy in the red cell, energy-dependent processes become impaired, especially the transport of sodium and potassium. It is likely that loss of ATP also is responsible for the decrease in red cell deformability that occurs with aging.<sup>45,54</sup>

Failure of the pentose phosphate pathway (Table 5-1) may also be an important factor in aging of the erythrocyte. When this step fails, the cell is unable to protect itself from oxidative damage. This may result in alterations in hemoglobin structure and function (Table 5-1). Ultimately, irreversible changes in hemoglobin may occur (page 104).

## Mechanisms of Red Cell Destruction

Which of the changes listed in Table 5-1 ultimately renders the erythrocyte nonviable remains unknown. Equally mysterious are the morphologic events accompanying red cell destruction. At least five major possible mechanisms of destruction have been defined, chiefly on the basis of either *in vitro* observa-

tions or study of exaggerated modes of destruction occurring in patients with hemolytic anemia. These mechanisms are (1) fragmentation, (2) osmotic lysis, (3) erythrophagocytosis, (4) complement-induced cytolysis, and (5) hemoglobin denaturation. As yet, the relative importance of each of these mechanisms in the destruction of normal cells remains unsettled.

### Fragmentation

Fragmentation may be defined as the loss of a portion of the erythrocyte membrane, often, but not always, accompanied by loss of cellular contents, including hemoglobin.<sup>98</sup> Fragmentation has been produced *in vitro* by a variety of techniques,<sup>98</sup> including microdissection,<sup>92</sup> thermal injury,<sup>80</sup> the forcing of cells through a micropipette of 2.5  $\mu$ m diameter,<sup>86,89</sup> or by the passage of cells through either a loose fibrin clot or an "artificial clot" made of nylon or glass fibers.<sup>73</sup> After a portion of the cell is lost, the membrane appears to be capable of self repair. In the intact organism, smaller fragments are probably removed from the circulation by reticuloendothelial cells; however, larger fragments may circulate, appearing on blood smears as small, misshapen, often triangular or "helmet-shaped" structures ("schistocytes"). Fragmentation is the characteristic mode of destruction in the "traumatic" and "microangiopathic" hemolytic anemias (Chapter 28) and is also a part of the destructive process in sickle cell anemia (Chapter 25) and the Heinz body anemias (Chapter 23).

That fragmentation is the chief means of destruction of normal cells was suggested by Rous and Robertson.<sup>92</sup> These investigators could find no morphologic signs of disintegrating, whole red cells (erythrophagocytosis) in the course of an organ-by-organ search of the body. They therefore concluded that, when the cell is deformed by passage through the microcirculation, portions are broken off until finally a fine, hemoglobin-containing dust is formed that is removed by the macrophages of the reticuloendothelial system. Some support for their hypothesis is found

Table 5-1. Changes in the Erythrocyte with Aging

- 1 Changes in glycolysis
  - a Enzyme activities which decrease
    - hexokinase<sup>34 36 37</sup>
    - phosphoglucose isomerase<sup>47</sup>
    - phosphofructokinase<sup>41</sup>
    - aldolase<sup>37</sup>
    - glyceraldehyde-3-phosphate dehydrogenase<sup>44</sup>
    - pyruvate kinase<sup>38 41</sup>
  - b Overall rate of glycolysis decreases<sup>33 38</sup>
  - c Phosphorylated intermediates which decrease
    - ATP<sup>37,44</sup>
    - 2,3 DPG<sup>33,42</sup>
- 2 Changes in the pentose phosphate pathways
  - a Glucose-6-phosphate dehydrogenase decreases<sup>34 35,37 47</sup>
  - b 6-Phosphogluconate dehydrogenase decreases<sup>35,37 47</sup>
- 3 Changes in the membrane
  - a Lipid content decreases<sup>52 55 56</sup>
  - b Surface area decreases<sup>52</sup>
  - c Negative charge decreases<sup>53</sup>
  - d Cellular cation content is altered K<sup>+</sup> decreases, Na<sup>+</sup> increases<sup>33,50</sup>
- 4 Activity of miscellaneous enzymes which decreases
  - Glutamate-oxaloacetate transaminase<sup>32 34 38 51</sup>
  - Cholinesterase<sup>32</sup>
  - Catalase<sup>31</sup>
  - Glyoxylase<sup>31</sup>
  - Isocitrate dehydrogenase<sup>34</sup>
  - Transketolase<sup>35</sup>
- 5 Changes in hemoglobin
  - a Increased methemoglobin<sup>44</sup>
  - b Increased hemoglobin A<sub>2</sub><sup>48</sup>
  - c Increased oxygen affinity<sup>40 42</sup>
- 6 Changes in physical properties
  - a Increased density<sup>39 49</sup>
  - b Decreased deformability<sup>45</sup>
  - c Increased osmotic and mechanical fragility<sup>43 47</sup>
- 7 Decreased cell size<sup>43 57</sup>

in the observation that the membrane becomes less and less deformable as the erythrocyte ages,<sup>45</sup> possibly because of depletion of ATP.<sup>54</sup> Such cells are more easily fragmented when deformed. In addition, the observation that the mean corpuscular volume and mean corpuscular hemoglobin decrease as the cell ages can only be explained by loss of a portion of the cell without total destruction.<sup>77</sup>

### Osmotic Lysis

Because of the osmotic effect of hemoglobin, erythrocytes tend to gain water; however, under normal circumstances this tendency is countered by an energy-dependent mechanism that pumps sodium and water out of the cell (page 100). If the rate of water entry exceeds the capacity of the pump, or if the operation of the pump is impaired, the cell gains water. Then the erythrocyte swells and changes in shape from a biconcave disc to a sphere. Normal erythrocytes can swell to as much as one and one half or two times their original volume without loss of contents.<sup>82, 96</sup> Further swelling, however, leads to the development of transient defects in the membrane, as great as 20 to 50 nm in diameter, through which hemoglobin and other macromolecules may pass.<sup>90</sup> This sequence of events is usually called osmotic or colloid-osmotic lysis; however, the term "lysis" is inexact since the membrane alteration is reversible, and cells that have undergone osmotic hemolysis can regain their osmotic integrity.

Osmotic lysis may be induced in vitro by exposure of cells to hypotonic saline solutions (the osmotic fragility test, page 734). Also, maneuvers that impair the function of the sodium pump may induce osmotic lysis.<sup>81</sup> Such maneuvers include inhibition of glycolysis—the source of the ATP that powers the pump—either by prolonged incubation in the absence of glucose or with chemical inhibitors such as fluoride. Similar effects can be obtained with agents acting on membrane ATPase, such as ouabain.

Increased membrane permeability (leakiness) is a consequence of certain kinds of red

cell damage<sup>84</sup> including the genetic abnormality of hereditary spherocytosis and some types of antibody damage (Chapter 27). Cells thus damaged may survive if they can compensate for their defect by increasing the rate of outward transport of sodium and water. However, if deprived of energy substrates, as may occur in the spleen, compensation fails and osmotic lysis ensues.

Spherical cells (spherocytes) are especially susceptible to osmotic lysis; therefore, this form of erythrocyte destruction is characteristic of hemolytic diseases accompanied by spherocytosis (Chapters 20, 21 and 27). Also, hereditary disorders of glycolytic enzymes may lead to failure of active transport and osmotic hemolysis. The importance of osmotic lysis in destruction of normal cells is unclear; however, depletion of glycolytic enzymes and increased osmotic fragility are characteristic changes in aged erythrocytes (Table 5-1).

### Erythrophagocytosis

Erythrophagocytosis refers to the ingestion of whole red cells by circulating monocytes or neutrophils or by fixed macrophages of the reticuloendothelial system. The phenomenon was probably first observed by Ehrlich, who placed a ligature around the finger of a patient with paroxysmal cold hemoglobinuria (page 915), chilled the finger, and collected blood from the fingertip.<sup>76</sup> Macrophages containing whole red cells were observed on microscopic examination.

When blood from normal subjects is incubated for one hour at 37° C, few of the phagocytic cells contained therein (less than 3/100,000) ingest red cells. In contrast, erythrophagocytosis is common in similarly treated blood from most patients with acquired hemolytic anemia.<sup>99</sup> The phenomenon appears to require that viable phagocytes be exposed to damaged erythrocytes,<sup>93, 97</sup> especially erythrocytes coated with complement-fixing antibodies.<sup>72, 78, 79</sup> Physiologic conditions of pH, temperature, and serum osmolality are required for optimal activity.

The process is blocked by metabolic inhibitors, especially iodoacetate, fluoride, dinitrophenol, and, to a lesser extent, cyanide.<sup>79</sup>

Erythrophagocytosis has been observed *in vivo*, especially in immunologic hemolytic anemias, but also in certain other forms of red cell injury.<sup>99</sup> Experimental induction of *in vivo* erythrophagocytosis has been accomplished by injection of antibodies directed against the red cell<sup>72</sup> or by damaging red cells in certain other ways.<sup>85,87</sup>

Erythrophagocytosis appears to be chiefly a pathologic phenomenon. Although it is often assumed to be an important mechanism for the disposal of normal, senescent red cells,<sup>90</sup> there is little direct evidence for this assumption. In one *in vitro* system employing allogeneic (mouse) macrophages, "old" human cells were phagocytized, but fresh ones were not<sup>97</sup>; however, the relation of this observation to the normal *in vivo* situation is uncertain. If all of the 200 billion normal red cells that are destroyed each day underwent erythrophagocytosis, one would expect to find morphologic evidence in careful examination of the RE system at autopsy.<sup>92</sup>

### Complement-Induced Cytolysis

One of the properties of complement (page 333) is the ability to attach to cells and induce lysis. The usual event that triggers cellular fixation of complement is the reaction between a cellular antigen and a humoral, "complement-fixing" antibody. In addition, complement is important in certain kinds of cell lysis apparently unrelated to antigen-antibody reactions, such as that which characterizes erythrocytes from patients with paroxysmal nocturnal hemoglobinuria (PNH) (Chapter 29).

The C8 and C9 components of complement appear to be the lytic agents. Although the exact nature of their interaction with the erythrocyte membrane is not known, the end result is the formation of pore-like defects in the membrane. Subsequent cell destruction proceeds by one of two mechanisms: (1) having become excessively permeable to water

and electrolytes, the cell undergoes osmotic lysis; or (2) hemoglobin and other constituents leak out of the cell directly without an intervening stage of osmotic swelling. *In vitro* models of the two mechanisms have been studied.<sup>94,95</sup> Lysis of the osmotic variety was produced by reacting human erythrocytes with complement and heteroimmune (rabbit) anti-erythrocyte serum.<sup>93</sup> Lysis in this system could be blocked by adding dextran of about 20,000 molecular weight to the medium. In contrast, anti-A antibodies induced non-osmotic hemolysis, and this could not be blocked with dextran. Lysis of erythrocytes from PNH was also of the latter type.<sup>94</sup> The explanation for the two modes of destruction remains controversial. In the view of one group of investigators,<sup>94,95</sup> the size of the membrane defects is the important factor. If they are smaller than 3.25 nm—the effective diffusion radius of hemoglobin—osmotic lysis ensues. If they are larger than 3.25 nm, hemoglobin can leak out without osmotic swelling. The effect of dextran, the molecular radius of which is about 3.2 nm, is explained by its action in countering the colloid osmotic pressure of hemoglobin. The factors controlling hole size are not completely understood, but appear to be a function of the nature or distribution of the antigen. Factors relating to the complement or the antibody are of little importance.

Another group of workers believes that it is the number of defects, rather than their size, that affects the type of hemolysis.<sup>75</sup> With the electron microscope, by means of a negative staining technique, this group demonstrated the complement-induced membrane defect. They found the defect to vary slightly in size with the source of complement (on the average, the size was 8.8 nm with guinea pig complement and 10.3 nm with human complement), but the size and nature of the holes did not differ with various types of antigen-antibody systems or in PNH. In contrast, the number of holes per cell varied considerably and could be manipulated *in vitro* by appropriate adjustments of the concentration of the C2 component of complement. However, hemolysis occurred even if

the average number of holes was as low as 1.73 per cell, as had been predicted previously on the basis of kinetic studies.<sup>88</sup> It was concluded that if the number of holes is large enough, as it might be in anti-A or PNH hemolysis, effectively larger defects might be produced by tearing between holes or by superimposition of two or more lesions. Such larger defects might permit the diffusion of hemoglobin.

There is little evidence that complement is involved in the destruction of normal, senescent erythrocytes. Sensitivity of cells to antibody-complement lysis was found to increase with cell age, but the differences were slight.<sup>91</sup>

### Hemoglobin Denaturation

The sequence of events occurring when erythrocytes are exposed to oxidant stress is discussed in Chapter 3 (page 104), along with the mechanisms serving to protect the cell from this type of damage. If these mechanisms fail, denatured hemoglobin precipitates, forming inclusion bodies known as Heinz bodies. Cellular destruction usually follows Heinz-body formation, partly because the cells containing these inclusions become sequestered in the spleen where complete or partial phagocytosis takes place. In addition, the Heinz bodies may form disulfide linkages with membrane sulfhydryl groups, a phenomenon associated with increased membrane permeability and increased susceptibility to osmotic lysis.<sup>83</sup>

Heinz-body formation is the principal mechanism of hemolysis in G6PD deficiency and related disorders (Chapter 23), in unstable hemoglobin disease (Chapter 24), and in certain of the thalassemias (Chapter 26). There is no solid evidence that Heinz-body formation is a preterminal event in the normal red cell; however, this possibility is suggested by the facts that aged cells are deficient in G6PD<sup>34,35,37,47</sup> and that they contain certain hemoglobin derivatives that are precursors of Heinz bodies.<sup>44,48</sup>

## Sites of Erythrocyte Destruction

Approximately 80 to 90% of normal erythrocyte destruction occurs without release of hemoglobin into plasma.<sup>117,118,131</sup> Because of this fact, the major part of the destructive process is presumed to be *extravascular*, probably within macrophages of the reticulo-endothelial (RE) system. Only 10 to 20% of normal destruction occurs *intravascularly*, and this mode of destruction has special characteristics to be discussed below. Most hemolytic anemias are characterized by predominantly extravascular destruction; in some, usually called hemoglobinurias, intravascular destruction predominates.

### Extravascular Hemolysis

For many years, it was considered that the RE cells of the liver play the major role in the breakdown of red cells. This theory arose because the experiments of Minkowsky and Naunyn were conducted in ducks and geese, animals in which the RE system is made up almost entirely of the hepatic Kupffer cells. In man the fixed macrophages of the RE system are more widely distributed. It is clear that other organs, especially the spleen, participate in erythrocyte destruction and that even in contused wounds, or in subcutaneous tissues into which erythrocytes have been injected, erythrocyte breakdown occurs.<sup>129</sup>

The relative importance of the spleen and liver in erythrocyte destruction is influenced by the degree of cell damage.<sup>90</sup> Severe degrees of damage lead to destruction in all parts of the RE system, but especially in the liver because of its relatively great blood flow. In contrast, when erythrocytes are injured less severely, destruction occurs in the spleen.<sup>74</sup> It is probable that effete red cells are destroyed primarily in the spleen; however, if this organ is removed from normal subjects, other parts of the RE system rapidly assume this function, and there is no increase in cell survival.<sup>135</sup> The erythroclastic func-

tion of the spleen and RE system is discussed in more detail in Chapter 8 (page 363).

### Intravascular Hemolysis

Special features characterize those situations in which red cells are destroyed within the circulation rather than within the RE cell. When this happens, hemoglobin is discharged directly into the circulation from which it is removed by several mechanisms (Fig. 5-4).

#### *Haptoglobin*<sup>118,121</sup>

At low rates of release of hemoglobin into plasma, all of the hemoglobin is found to be attached to haptoglobin. This specific, hemoglobin-binding protein was first detected in plasma by its ability to enhance the peroxidase activity of hemoglobin.<sup>134</sup> Since its concentration was found to increase in a variety of inflammatory diseases, this was recognized as a nonspecific sign of disease with much the same significance as an accelerated sedimentation rate (page 127). The role of haptoglobin as a hemoglobin-binding protein and as the principal factor affecting the "renal threshold" for hemoglobin was described by Laurell and Nyman.<sup>124</sup>

Haptoglobin is an  $\alpha_2$ -glycoprotein. By starch gel electrophoresis, a number of haptoglobin bands may be demonstrated. The observed pattern reflects the genetic constitution.<sup>139</sup> There are two principal, allelic, autosomal genes, Hp1 and Hp2, neither of which is dominant. These result in three principal phenotypes, Hp1-1, Hp2-2, and Hp2-1 (Fig. 5-5). The incidence of these phenotypes in white Americans is 15%, 38%, and 47%, respectively. There also are subgroups of these basic types; two normal varieties of the Hp1 gene have been distinguished, namely, Hp1F and Hp1S. Rare genetic variants of the common genes also have been reported.<sup>115,121</sup> The haptoglobin in individuals of phenotype Hp1-1 is a single molecular species with a molecular weight of about 85,000. The molecule resembles that of certain immunoglobulins in that it has two

"light" ( $\alpha$ ) chains and two "heavy" ( $\beta$ ) chains. In individuals of phenotype Hp2-2 and Hp2-1, a number of haptoglobin bands are found (Fig. 5-5), and these appear to be polymers of a basic subunit similar in size to Hp1-1. The genetic variation affects the "light" ( $\alpha$ ) chains only; the heavy chains appear to be identical in all phenotypes.

The normal plasma haptoglobin concentration is  $1.28 \pm 0.25$  g/l.<sup>122</sup> More often, the concentration is expressed in terms of the hemoglobin-binding capacity. The normal values for the latter differ with phenotype: for Hp1-1,  $1.36 \pm 0.37$  g/l; for Hp2-1,  $1.08 \pm 0.37$  g/l; and for Hp2-2,  $0.82 \pm 0.34$  g/l.<sup>132</sup> If phenotype is disregarded, the normal range is 0.4 to 2.0 g/l.<sup>118</sup> The association constant between haptoglobin and hemoglobin is so great that the reaction may be considered irreversible. Haptoglobin (Hp) is able to bind oxyhemoglobin (Hb), methemoglobin, isolated Hb  $\alpha$ -chains,  $\alpha\beta$  dimers and heme-free globin, but it will not bind deoxygenated hemoglobin, myoglobin, heme, hemoglobin H or Barts, or isolated Hb  $\beta$ -chains. The binding site on Hb is thought to be on the  $\alpha$ -chain and the binding site on Hp is probably on its heavy ( $\beta$ ) chain.<sup>119</sup> The chemical nature of the bond is unknown.

In most instances, the molar ratio of Hp to Hb in the HpHb complex is 1:1 if a reference molecular weight of 85,000 for haptoglobin is used to correct for polymerization.<sup>141</sup> It has been observed that with horse Hb at least three different types of HpHb complexes are formed in vitro and the relative proportions of each depend on the ratios of hemoglobin and haptoglobin present.<sup>141</sup> These three complexes, which differ in the degree to which the Hb molecule has dissociated, have been designated HpHb, Cx, and Cd. In HpHb, one Hb  $\alpha\beta$  dimer, one Hb  $\alpha$ -chain, and one Hb  $\beta$ -chain are bound; in Cx, two  $\alpha$ -chains and two  $\beta$ -chains are bound; and in Cd, one  $\alpha$ - and one  $\beta$ -chain are bound. Others, however, have found that if only human hemoglobin is added, a comparable series of complexes is not found;

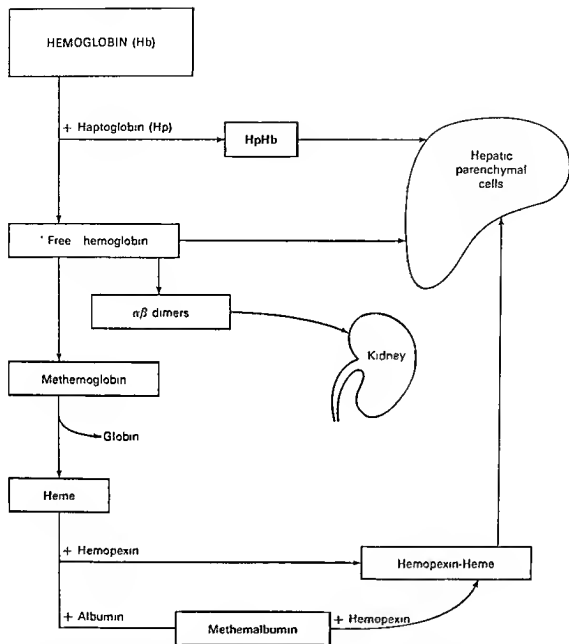


Fig 5-4 Pathways for the disposal of hemoglobin (Hb) in plasma. The initial pathway is through binding by haptoglobin (Hp) with subsequent removal of the HbHp complex by hepatic parenchymal cells.<sup>123</sup> Hemoglobin in excess of the Hp binding capacity circulates as the unbound (free) protein. In this form it is partially removed by hepatic cells, but it may also follow two other pathways. It may dissociate into two half molecules ( $\alpha\beta$  dimers) that are excreted by the kidney. In addition, it may be oxidized to methemoglobin, from which heme is easily dissociated. The latter is initially bound to hemopexin, which transports it to the hepatic parenchymal cell. Heme may also be bound nonspecifically by albumin, forming methemalbumin. This complex transfers its heme to hemopexin as the latter becomes available.

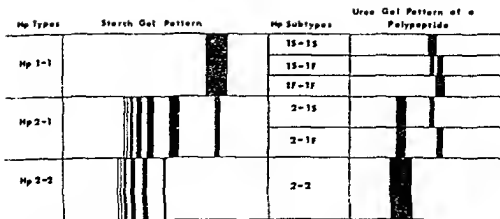


Fig 5-5. Starch gel electrophoretic patterns of the common haptoglobin (Hp) phenotypes. The three major phenotypes (left) can be subdivided on the basis of analysis of the light (a) polypeptide (right). (From Giblett,<sup>118</sup> courtesy of the author and Williams & Wilkins Company.)

instead, two  $\alpha\beta$  dimers are bound symmetrically to each Hp molecule.<sup>127</sup>

Haptoglobin is synthesized in the parenchymal cells of the liver.<sup>112,128</sup> When not bound to hemoglobin, it leaves the plasma with a half-disappearance time of about five days. The HpHb complex leaves much more rapidly with a half-disappearance time of about nine minutes. About 50 to 80% of the haptoglobin turnover in the normal subject is accounted for by the rapid pathway.<sup>131</sup> The hepatic parenchymal cell appears to be the main site of removal of the HpHb complex.<sup>113</sup> From the kinetics of haptoglobin turnover it may be calculated that some 10 to 20% of normal erythrocyte destruction occurs intravascularly and utilizes the haptoglobin system.<sup>118,131</sup> Since a portion of any unbound hemoglobin is excreted into the urine, it is reasonable to infer that the normal physiologic function of haptoglobin is to prevent renal loss of hemoglobin, thereby conserving iron and protecting the renal tubular cells from damage.<sup>111</sup> In hemolytic anemias characterized by intravascular hemolysis, catabolism of Hp is so rapid that it essentially disappears from the plasma, a change that is not accompanied by a compensatory increase in haptoglobin synthesis. Hypohaptoglobinemia may also be observed in certain hemolytic states associated with predominantly extravascular destructive mechanisms.<sup>118</sup> The explanation for this is

not clear, but it has been suggested that some hemoglobin may be regurgitated from RE cells when the rate of phagocytosis of erythrocytes or erythrocyte fragments reaches a maximum.<sup>158</sup>

#### *Hemoglobin and the Kidney<sup>133</sup>*

The hemoglobin-haptoglobin complex is too large (molecular weight about 150,000) to pass through the glomerulus. Thus, the level of circulating haptoglobin is the most important determinant of the apparent renal threshold.<sup>121</sup> When the haptoglobin system is saturated, free (unbound) hemoglobin circulates briefly in plasma. The hepatic parenchymal cell is partially responsible for the disposal of free hemoglobin,<sup>113</sup> but, in addition, hemoglobin dissociates into two  $\alpha\beta$  dimers which, having a molecular weight of about 32,000, readily pass through the glomerulus.<sup>113</sup> There is a low (0.2 to 0.6 g/l) renal threshold for free hemoglobin that is related to renal tubular reabsorption rather than to haptoglobin.<sup>123</sup>

Much of the hemoglobin appearing in the glomerular filtrate is reabsorbed in the proximal tubule.<sup>123</sup> The rate of tubular reabsorption of hemoglobin in adult males is  $1.43 \pm 0.96$  mg/min.<sup>126</sup> If this capacity is exceeded, hemoglobin appears in the urine. Thus, renal handling of hemoglobin is similar



to that of glucose, urate, and certain other substances.<sup>115</sup>

Within the tubular epithelial cell, hemoglobin iron is rapidly extracted and stored in the cell as ferritin and hemosiderin.<sup>115,136</sup> Some of the tubular epithelial iron may be reutilized for hemoglobin synthesis, but only at a very slow rate.<sup>115</sup> When iron-laden tubular cells are sloughed into the urine, the urine iron concentration increases and both ferritin and hemosiderin may be detected.<sup>136</sup>

### Plasma Heme

Free hemoglobin in plasma may be readily oxidized to methemoglobin. The latter dissociates easily and nonenzymatically into heme and globin.<sup>114</sup> Free heme is highly insoluble at physiologic pH, but two serum proteins, hemopexin and albumin, are able to bind it and maintain it in a soluble form. *Hemopexin*,<sup>130</sup> a  $\beta_1$ -globulin, is a glycoprotein with a molecular weight of about 70,000, of which 20% is carbohydrate. It is synthesized in the liver<sup>140</sup> and is found in the plasma of adults in a concentration of 50 to 100 mg/dl. Each hemopexin molecule binds one molecule of heme and may also bind certain porphyrins, but not bile pigments. The hemopexin-heme complex is removed from the circulation by the hepatic parenchymal cell, with a half-disappearance time of seven to eight hours.<sup>130</sup> Like haptoglobin, hemopexin is depleted as it serves its function.<sup>130,137</sup> Plasma hemopexin values may be reduced in conditions associated with intravascular hemolysis, but the depletion is less regular and less pronounced than that of haptoglobin. Especially low hemopexin values are found in thalassemia major and sickle cell anemia.<sup>130</sup>

Albumin binds heme in a 1:1 molar ratio<sup>125</sup> to form methemalbumin.<sup>116</sup> The affinity of albumin for heme is much less than that of hemopexin.<sup>130</sup> Methemalbumin may be detected by its spectral properties and by the formation of a hemochromogen with a sharply defined absorption band at 558 nm in the presence of ammonium sulfide (Schumm's test). This test was formerly considered to be specific for methemalbumin;

however, hemopexin-heme forms a similar chromogen.<sup>135</sup> The disappearance of methemalbumin from the circulation is kinetically complex, but it is clearly slower than that of hemopexin-heme.<sup>137</sup> It is possible that methemalbumin follows no specific disposal pathway, but, instead, gradually transfers its heme to hemopexin as the latter becomes available.<sup>130</sup>

## Hemoglobin Catabolism<sup>178,191</sup>

### Formation of Bilirubin

About 30 minutes after injection of either hemoglobin or nonviable red cells, there is an increase in bilirubin in serum and bile as well as in iron entering plasma and in carbon monoxide, derived from the  $\alpha$ -methene carbon of heme,<sup>159,160</sup> exhaled from the lungs. Studies of the nonenzymatic oxidation of hemoglobin with oxygen and ascorbate led Lemberg to believe that the first step in hemoglobin catabolism was the opening of the porphyrin ring by oxidation of the  $\alpha$ -methene bridge, the iron remaining and the union with globin persisting to form a green, iron protein compound, verdohemoglobin or choleglobin.<sup>176,177</sup> The discovery of an enzyme system that converts heme to bilirubin has led to revision of this concept.<sup>191</sup>

The enzyme, microsomal heme oxygenase, catalyzes the degradation of heme to iron, carbon monoxide, and a greenish pigment, biliverdin (Fig. 5-6). Since the substrate for this reaction is heme, not hemoglobin, dissociation of heme from globin must be presumed to precede the reaction. The enzyme is found in the microsomal fraction of homogenates of a number of organs. Activity is greatest in spleen, followed by bone marrow, liver, brain, kidney, and lung. Microsomal heme oxygenase appears to be inducible by its substrate, since activity is markedly increased by the prior injection of methemalbumin as well as by injection of hemoglobin or by induction of hemolytic anemia. Also, splenectomy is followed by a two- to three-fold increase in enzyme activity in the liver,<sup>194</sup> and hemoglobinuria is associated

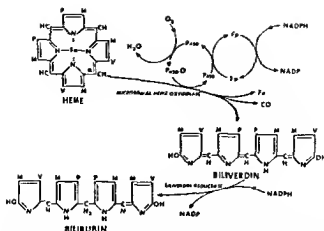


Fig. 5-6. Enzymatic degradation of heme to form iron (Fe), carbon monoxide (CO), and bilirubin. The porphyrin ring is cleaved by oxidation of the  $\alpha$ -methene bridge (a). The reaction depends upon an enzyme, microsomal heme oxygenase, and requires oxygen and nicotinamide adenine dinucleotide phosphate (NADPH) as cofactors. In addition, an abbreviated electron transport system is utilized, which includes cytochrome P-450 ( $P_{450}$ ) and a flavoprotein (Fp). Biliverdin is reduced to bilirubin by NADPH in a reaction catalyzed by biliverdin reductase. Side chains of heme and the bile pigments are abbreviated as follows: M, methyl; V, vinyl; P, propionate. (From Tenhunen et al.<sup>194</sup> courtesy of the authors and Transactions of Association of American Physicians.)

with induction of the enzyme in renal tubular cells.<sup>133</sup> Enzyme induction has also been observed in vitro in peritoneal macrophages.<sup>78</sup> In such preparations, heme oxygenase activity increased dramatically about three hours after erythrophagocytosis. Induction was blocked with puromycin, actinomycin D, and hydrocortisone, but the hydrocortisone effect could be prevented with glucose and insulin.

The heme oxygenase reaction is coupled with a second step in which biliverdin is reduced to bilirubin (Fig. 5-6). This reaction is catalyzed by a soluble, NADPH-dependent enzyme, biliverdin reductase. The system converting heme to bilirubin is classed with a group of similar enzymes termed "mixed-function" oxidases, which are characterized by the utilization of an abbreviated, microsomal electron transport system with cytochrome P-450 as the terminal oxidase. The stoichiometry of the reactions is such that three moles of oxygen and probably four moles of NADPH are utilized for each mole of bilirubin and carbon monoxide formed.

The bilirubin formed in vitro by this sys-

tem is of the isomer type that occurs naturally in vivo. This isomer is designated IX  $\alpha$  to indicate that it has the side chain structure of protoporphyrin IX and that cleavage of the porphyrin ring takes place at the  $\alpha$ -methene bridge. In contrast, a mixture of bilirubin isomers is synthesized in the system described by Lemberg.<sup>191</sup> The only known endogenous source for carbon monoxide is in the microsomal heme oxygenase reaction.<sup>160</sup> Consequently, measurement of endogenous carbon monoxide production may be used as a measure of the rate of red cell destruction (page 216).

If isotopically labeled glycine is administered to a normal person, the label is incorporated into the porphyrin ring and ultimately makes its appearance in bilirubin<sup>140</sup> (Fig. 5-7). The label appears in about 85% of bilirubin at about 120 days after administration of the isotope and therefore is presumed to be derived from the destruction of senescent red cells; the remaining 15% of the pigment is labeled within several days and hence is often referred to as the "early-

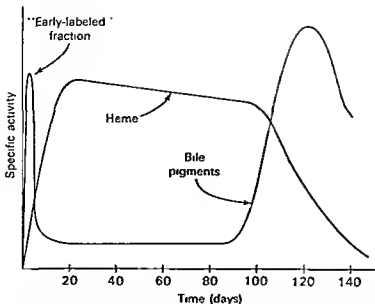


Fig 5-7. The incorporation of isotopically labeled glycine into heme and bile pigments. Most of the bilirubin labeling occurs at about 120 days, at the time of destruction of senescent red cells. However, about 15% of bilirubin is labeled within the first several days and is therefore called the "early-labeled" fraction.

labeled" bilirubin. This fraction is made up of at least two components.<sup>172,190</sup> The first, normally the larger one, is labeled maximally at 24 hours and is of hepatic origin, probably chiefly from the heme of cytochrome P-450. The second is labeled maximally at about three to four days and is a by-product of erythropoiesis.<sup>154,172</sup> An isotopic label from administered aminolevulinic acid appears primarily in the hepatic fraction, whereas that from glycine appears in both.<sup>190</sup> The erythropoietic component is probably the product of "ineffective erythropoiesis" (page 550), and results from the intramedullary destruction of defective red cells. In illnesses associated with exaggerated ineffective erythropoiesis, such as thalassemia or pernicious anemia, there is a marked increase in the "early-labeled" bilirubin fraction.

### Bilirubin Transport

After being released from the sites of heme catabolism, bilirubin appears in plasma. The normal value for plasma bilirubin is usually given as 0.5 to 1.0 mg/dl; however, some

studies have suggested that the distribution of values in a normal population is skewed and up to 1.5 mg/dl may be within normal limits.<sup>196</sup> Being poorly soluble in water, bilirubin carried in plasma must be bound to albumin, which greatly increases its solubility. Each molecule of albumin is able to bind two molecules of bilirubin, the first molecule being more tightly bound than the second.<sup>184,186</sup> At normal plasma albumin concentrations, the theoretical bilirubin-binding capacity is of the order of 70 mg/dl, of which half, or 35 mg/dl, is tightly bound. These values are reduced by a decrease in plasma albumin concentration or by the presence of organic, anionic substances that compete for albumin-binding sites, such as heme, fatty acids, sulfonamides, and salicylates. When the binding capacity is exceeded, bilirubin diffuses into the tissues, especially tissues with a high lipid content.

Under certain pathologic circumstances, bilirubin diglucuronide (conjugated bilirubin, "direct-reacting" bilirubin) is found in the plasma. This relatively soluble bilirubin derivative may also be bound to albumin, but

less tightly so than the unconjugated form. Some of the plasma bilirubin diglucuronide is ultrafilterable, being bound to an unidentified material of low molecular weight.<sup>166,167</sup> This complex passes through the glomerulus, is not reabsorbed in the tubule, and thus is excreted into the urine. In contrast, unconjugated bilirubin is not excreted by the kidney.

### Hepatic Bilirubin Metabolism

The processing of bilirubin by the liver may be thought of as only one aspect of a general mechanism whereby plasma protein-bound, organic anions are metabolized

and excreted. In addition to bilirubin, other anionic substances follow the same pathways, especially certain dyes (eg, bromosulfalein or BSP), cholecystographic agents, drugs (eg, salicylates), steroids, and thyroxine. The process may be divided into three distinct phases: uptake, conjugation, and excretion<sup>151</sup> (Fig. 5-8). All three phases must be operative if bilirubin is to be excreted at a normal rate; however, in the normal person the excretion step is the slowest, and therefore the rate-limiting, step.

Relatively little is known about the mechanism of hepatic *bilirubin uptake*. However, it is clear that the process is rapid and efficient. In rats, for example, 65% of an admin-

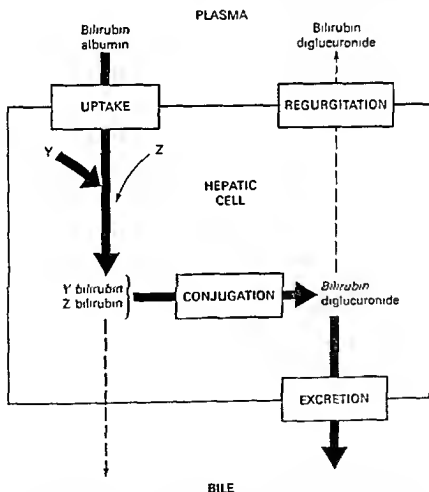


Fig 5-8. Normal and abnormal pathways of bilirubin excretion by the hepatic cell. The normal pathways (solid arrows) include uptake and conjugation of bilirubin and excretion of the conjugated derivative. Abnormal pathways (dashed arrows) include regurgitation of bilirubin diglucuronide into plasma and excretion of unconjugated bilirubin into bile. Y and Z are bilirubin-binding proteins that may play a role in the uptake step.

istered dose of bilirubin is taken up by the liver within five minutes. There are two viewpoints regarding the mechanism of hepatic uptake. One of these holds it to be a function of the hepatocyte plasma membrane, and to be a carrier-mediated, active transport process, perhaps involving specific membrane receptor sites.<sup>178</sup> To date, there is little evidence to support or refute this hypothesis. However, the discovery of two bilirubin-binding proteins, designated Y and Z, in hepatocyte cytoplasm<sup>179</sup> has led to the alternate proposal that hepatic uptake is a function of these intracellular binding proteins.<sup>152</sup> Y protein, which has been isolated in pure form, constitutes about 5% of hepatic, cytoplasmic protein.<sup>152</sup> Structurally, it is a 44,000 molecular weight dimer formed from two identical 22,000 molecular weight subunits. Z protein, which has been only partially purified, is present in smaller amounts than Y; it has a molecular weight of 9000 to 10,000. Administration of certain drugs, notably phenobarbital, brings about a threefold increase in the concentration of Y, but Z is unaffected.<sup>188</sup> Both Y and Z proteins bind many organic anions in addition to bilirubin.<sup>152</sup> It is proposed that bilirubin, bound to albumin, is presented to the hepatocyte membrane, where it dissociates from albumin and crosses the membrane by non-ionic diffusion. A rapid, bidirectional flux between plasma and liver ensues,<sup>168</sup> the net hepatic uptake being determined by the amount and binding affinity of albumin in the plasma and the amount and binding affinity of Y and Z protein in the cytoplasm.<sup>152</sup> The unconjugated hyperbilirubinemia that occurs in patients given flavaoidic acid, an agent used in treatment of certain tapeworm infestations, has been ascribed to competition between the drug and bilirubin for binding sites on the Z protein.<sup>152,183</sup> On the basis of indirect evidence, it has been suggested that the abnormality in a proportion of patients with Gilbert's syndrome<sup>189</sup> (idiopathic unconjugated hyperbilirubinemia) is impaired hepatic uptake of bilirubin.<sup>156</sup> As yet, the amounts and binding affinities of Y and Z proteins in this syndrome have not been investigated.

Having entered the liver cell, bilirubin is *conjugated* with two molecules of glucuronic acid to form bilirubin diglucuronide. The reaction is catalyzed by uridine diphosphate (UDP)-glucuronyl transferase, an enzyme associated with the endoplasmic reticulum membrane.<sup>152,175</sup> Proliferation of endoplasmic reticulum and markedly increased activity of UDP-glucuronyl transferase occur after administration of phenobarbital and certain other drugs.<sup>203</sup> In the conjugation reaction, glucuronic acid, a derivative of glucose, is transferred from UDP-glucuronic acid to the propionyl carboxyl groups of bilirubin to which it becomes attached through ester linkages, forming bilirubin diglucuronide. It is uncertain whether all these reactions are catalyzed by the same enzyme system or if there are several glucuronyl transferases. Traces of bilirubin may be conjugated with sulfate<sup>173</sup> rather than glucuronide, but little is known about this pathway and it is not likely to be of functional importance.<sup>178</sup> Bilirubin diglucuronide is considerably more polar and water-soluble than unconjugated bilirubin. In hepatocellular disease or biliary obstruction, it may be "regurgitated" into plasma. Normally, it is excreted into the bile. Little or no unconjugated bilirubin is found in bile, and if conjugation does not occur, bile bilirubin content is very low. Hereditary lack of UDP-glucuronyl transferase leads to severe jaundice in the "Gunn" rat.<sup>157,170</sup> It is probable that the rare, human illness, the Crigler-Najjar syndrome,<sup>161</sup> is the human equivalent of the same defect.<sup>167,178</sup> One form of Gilbert's syndrome may result from mild UDP-glucuronyl transferase deficiency.<sup>153</sup> Jaundice in this syndrome is relieved by phenobarbital, presumably because of its effects on enzyme concentration.

*Excretion* of conjugated bilirubin from the hepatic cell into the bile canaliculus is generally considered to be an active transport process, since it proceeds against large concentration gradients. It is the least understood aspect of hepatic bilirubin metabolism. Neither the energy source nor a specific carrier has been identified.<sup>152</sup> It has been suggested

that the Golgi apparatus is involved.<sup>152</sup> The Dubin-Johnson syndrome<sup>162</sup> probably results from a defect in cellular excretion of bilirubin and certain other organic anions.<sup>151,178</sup>

### Intestinal Bile Pigment Metabolism<sup>163,178</sup>

Bilirubin diglucuronide is excreted into the duodenum with other constituents of bile. There is little or no intestinal absorption of the conjugated pigment, although unconjugated bilirubin is readily absorbed. Bilirubin diglucuronide probably remains in the conjugated form until it reaches the terminal ileum and colon where bacterial  $\beta$ -glucuronidases hydrolyze it. The two methene ( $-\text{CH}=\text{}$ ) bridges and usually the two vinyl groups are then reduced by bacterial flora to form a series of compounds called *urobilinogens*<sup>197</sup> (Fig. 5-9). These are colorless compounds that react with Ehrlich's aldehyde reagent to form a red-violet chromogen. Since urobilinogen formation is accomplished by bacteria, it does not occur in newborn or

in germ-free animals,<sup>171</sup> and it may be markedly affected by administration of broad-spectrum antibiotics.<sup>197</sup> The urobilinogens are easily dehydrogenated across the two middle rings to form *urobilins* (Fig. 5-9). The latter differ from urobilinogens in that they are colored (orange-yellow) and do not react with Ehrlich's reagent. Urobilins are characterized further by their ability to react with zinc to form fluorescent (green) compounds (Schlesinger reaction).

About 10 to 20% of the urobilinogen formed in the gut is reabsorbed and the remainder is lost with the feces. The reabsorbed fraction is efficiently excreted by the normal liver without being conjugated.<sup>178</sup> This sequence of events is referred to as the enterohepatic recirculation of urobilinogen. A portion of the reabsorbed pigment may also be excreted into the urine. Urobilinogen is filtered by the glomerulus, secreted by the renal tubule, and reabsorbed. Tubular reabsorption is facilitated in acid urine; therefore, renal clearance is greatest when the

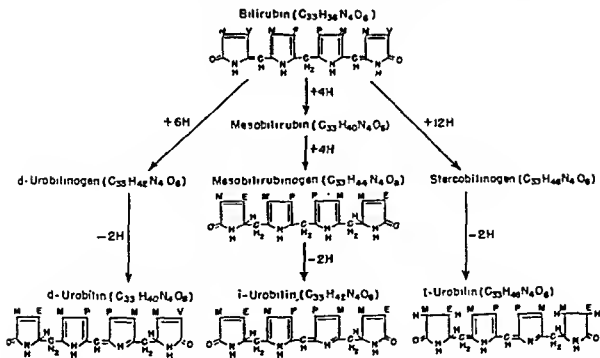


Fig 5-9. Bile pigment metabolism in the gut. These reactions are carried out by bacteria. d-Urobilinogen, mesobilirubinogen and stercobilinogen are members of the urobilinogen group, which is characterized structurally by saturation of the carbon bridges connecting the pyrrole rings. The urobilins are derived from urobilinogens by oxidation and have one or more double bonds in the connecting carbon bridge. (From Lester and Troxler,<sup>178</sup> courtesy of the authors and Gastroenterology.)

urine is alkaline. If the liver's capacity to excrete urobilinogen is impaired a disproportionate amount appears in the urine.

Also found in the colon are a group of poorly characterized dipyrroles known as mesobilifuscins. These brown pigments are partly responsible for the color of normal feces.<sup>202</sup> Most of these dipyrroles do not appear to be derived from the degradation of bilirubin; instead, they probably are anabolic by-products of heme synthesis.<sup>168</sup> However, in conditions associated with Heinz-bodies (Chapters 23, 24, and 26), excessive amounts of such dipyrroles are excreted in the urine. These are thought to be derived from the heme released when the Heinz body forms (page 817). Furthermore, a dipyrrole is formed from the photodegradation of bilirubin,<sup>183</sup> as discussed in the following section.

#### Alternate Pathways of Heme and Bilirubin Catabolism

On a strictly stoichiometric basis, it may be calculated that 35 mg of bilirubin should be produced from the quantitative destruction of 1 g of hemoglobin. Assuming a normal red cell survival of 120 days, the daily excretion of bilirubin and urobilinogens should be 28 mg per 100 g circulating hemoglobin per day, even if there were no contribution from nonerythroid pathways. In normal subjects, however, only 11 to 21 mg/100 g hemoglobin/day are excreted.<sup>182</sup> These observations imply that as much as 20 to 40% of heme may be degraded by pathways other than bilirubin,<sup>198</sup> or that bilirubin itself may be converted to catabolites other than urobilinogen or urobilin.

The existence of such alternate pathways was demonstrated experimentally when only 60 to 80% of the radioactivity in administered labeled heme, hemoglobin, or damaged erythrocytes could be accounted for as bilirubin collected from rats with biliary fistulas.<sup>174,187</sup> Radioactivity in unidentified, non-bilirubin components of bile was found in such animals.<sup>174</sup> Furthermore, in severe, inherited defects of bilirubin conjugation, such as those

found in the Gunn rat or in human infants with the Crigler-Najjar syndrome, the alternate pathways appear to be increased.<sup>192</sup>

It is possible that some of these observations can be explained by a series of reactions whereby bilirubin is converted to a variety of water-soluble derivatives, including hydroxyrubins, bilichrysin, and a dipyrrole.<sup>185</sup> These conversions are accompanied by loss of yellow color and diazo reactivity, and are accelerated in vitro and in vivo by exposure to light. Similar or identical reactions occur in vitro in the dark in alkaline, aqueous solutions. Products of the reaction are found in the bile of the Gunn rat.

These photodegradation reactions of bilirubin have been applied to the treatment of unconjugated hyperbilirubinemia in infants.<sup>181</sup> Exposure to light brings about a prompt decrease in serum bilirubin concentration to non-toxic levels. The photodegradation products are excreted promptly in the bile and may cause the stool to turn to a green or darker brown color. No toxicity of these products has been demonstrated, but only short-term observations are currently available.

#### Laboratory Evaluation of Hemoglobin Catabolism and Bile Pigments

##### *Icterus Index (Meulengracht Test)*

Determination of the icterus index is a very simple procedure for the rough quantitation of the degree of yellowish coloring of the plasma or serum. Comparison is made with the color of standard solutions of potassium dichromate made up in a series of tubes numbered according to the quantity of dichromate in 10,000 parts of water; thus, 1 unit is 1:10,000, 5 is 5:10,000, and so on.

The simplest procedure is to compare the color of the plasma, as seen in the Wintrobe hematocrit after it has been centrifuged, with that of a series of standards in tubes of glass of the same thickness and bore as the hematocrit (Fig. 5-10 and Frontispiece). If the icterus index is greater than 100, the plasma may be diluted until a match is obtained.

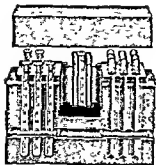


Fig. 5-10. Icterus index and blood sedimentation apparatus for use in conjunction with hematocrit. The standard icterus index tubes are shown on the right. The unknown is matched against the standards in the central portion of the block. The section at the front of the block is for holding hematocrits vertically during sedimentation.

The icterus index is a useful, simple, and rapid test. When it is normal (5 to 7.5 units) it can be assumed that there is no increase in the bilirubin content of the blood stream. The test is important during a routine blood examination in calling attention to the presence of unsuspected hyperbilirubinemia. It must be borne in mind that it is not specific and that substances other than bilirubin may cause an increase in the yellow color. Of these substances the lipochromes (carotene, lutein, etc.) are the most important. Thus, in vegetarians, diabetics, infants, and patients with myxedema there may be an increased icterus index that is not the result of hyperbilirubinemia. The drug atabrine may occasionally be sufficiently concentrated in the blood to cause an elevation in the icterus index.

#### *van den Bergh Test*

As distinguished from the icterus index, the van den Bergh test is a specific test for bilirubin. It is based on Ehrlich's discovery that a mixture of sulfanilic acid, hydrochloric acid, and sodium nitrite (diaz reagent) yields a reddish-violet color with a maximum absorption at a wavelength of 430 nm when added to plasma or other solutions containing bilirubin. The color may appear and reach its maximum intensity at once ("direct" reaction); if no color develops in one minute, alcohol is added. If the color then appears,

the reaction is called "indirect." In general, unconjugated bilirubin is indirect-reacting and bilirubin diglucuronide is direct-reacting. However, because of the kinetics of the van den Bergh reaction and the presence of solubilizing substances in the plasma, the direct and indirect test yields only approximations of the respective concentrations of bilirubin diglucuronide and of bilirubin. The difference between the reactivity of conjugated and unconjugated bilirubin has been ascribed to relative water solubility of the two compounds; however, a more plausible explanation is the difference in hydrogen bonding within the molecules and the resulting steric interference with access of the diazo reagent to the central methene group.<sup>165</sup>

#### *Tests for Urobilinogen and Urobilin*

Practical tests for urobilinogen and urobilin are based on Ehrlich's aldehyde reaction. When Ehrlich's reagent (paradimethylaminobenzaldehyde) reacts with a member of the urobilinogen group, a red-violet chromogen (maximum absorption wavelength, 565 nm) is formed. Urobilin is Ehrlich negative, but if it is first reduced to urobilinogen with ferrous sulfate in sodium hydroxide, a positive reaction is obtained. The reaction is not specific for urobilinogen.<sup>163</sup> Monopyrroles, such as porphobilinogen (page 170), indoles, and certain other substances form similar pigments. In addition, some indicator dyes (eg, pyridium) turn red when exposed to the acid in Ehrlich's reagent.

Semiquantitative assays for urobilinogen that are of practical value have been devised by Watson et al.<sup>209</sup> For serial observations of urine urobilinogen, a two-hour sample is collected between 2 and 4 P.M. following emptying of the bladder and drinking one glass of water. During this period of the day the peak excretion of urobilinogen usually occurs. The sample is cooled to room temperature and examined within 30 minutes after voiding. Normal values for the two-hour period range from 0.1 to 1.5 "Ehrlich units," which are approximately equivalent to 1 mg of urobilinogen. The term "unit" is



used because the method is not specific for urobilinogen; other Ehrlich-reacting substances are measured as well. The increase of these other substances has been found to be roughly proportional to that of urobilinogen and consequently the test is useful for serial observations. It has been found to give false negative results in about 15% of patients with significant degrees of hyperurobilinogenuria.<sup>195</sup>

A *semiquantitative method* may also be used on random fecal specimens.<sup>200</sup> The upper limit of normal is about 350 Ehrlich units per 100 g. The *quantitative method* is discussed on page 217.

### *The Rate of Heme Catabolism*

As discussed elsewhere in this chapter (page 209), the principal catabolic products of heme are iron, carbon monoxide, and bilirubin. There are no other significant endogenous sources of the last two compounds; thus, the breakdown of one mole of heme yields precisely one mole of carbon monoxide and one mole of bilirubin. Measurements of the endogenous production of carbon monoxide or bilirubin constitute accurate assessments of the rate of heme catabolism. The methodology is complex, however, and, for the most part, such measurements have been performed in research settings. More available to clinicians is the determination of fecal urobilinogen excretion—an older, less accurate approach to quantitation of heme breakdown.

From any of these measures of the rate of heme catabolism together with the determined or estimated circulating red cell volume, the erythrocyte life span can be estimated.<sup>155</sup> Such estimates are based on the assumption that about 85% of the heme broken down comes from the destruction of circulating red cells (page 209). This assumption is valid except in those conditions associated with ineffective erythropoiesis. Consequently, the demonstration that the amount of heme catabolized exceeds that which can be accounted for on the basis of a simultaneously performed study of erythro-

cyte life span constitutes excellent evidence for ineffective erythropoiesis.

**ENDOGENOUS CARBON MONOXIDE (CO) PRODUCTION.** Sjostrand<sup>193</sup> and, later, Engstedt<sup>164</sup> called attention to the fact that blood carboxyhemoglobin (COHb) levels are increased in hemolytic disease. Precise interpretation of such static measurements is complicated by exogenous exposure to CO and by variations in CO excretion. In contrast, however, the endogenous rate of CO production ( $\dot{V}_{CO}$ ) can be measured by a re-breathing method that circumvents these problems.<sup>160</sup> The subject breathes into a closed system from which CO<sub>2</sub> is absorbed and to which O<sub>2</sub> is added. CO excretion is thereby prevented and the blood level of COHb increases. Endogenous CO production is calculated from the rate of increase over a two-hour period and from the body CO dilution. The latter is determined by adding a small quantity of CO to the re-breathing system and measuring the resultant increase in blood COHb.

With this method, the average normal value for  $\dot{V}_{CO}$  was found to be  $18.7 \pm 3.2$  (SD)  $\mu\text{mol/hr}$ . In eight patients with various types of hemolytic anemia, the values ranged from 31 to 143  $\mu\text{mol/hr}$  or about 1.5 to 8 times normal.<sup>159</sup> In five subjects with conditions associated with ineffective erythropoiesis, values from 21 to 94  $\mu\text{mol/hr}$  were found.<sup>201</sup>

**BILIRUBIN PRODUCTION.** The rate of bilirubin production can be calculated from the kinetics of plasma bilirubin disappearance.<sup>153</sup> A tracer dose of <sup>3</sup>H- or <sup>14</sup>C-bilirubin is injected intravenously, the bilirubin pool size is measured, and serial determinations of plasma unconjugated radioactive bilirubin are made over a 24- to 48-hour period. The disappearance curve is complex and requires analysis by digital computer. The curve appears to be the sum of three exponential components with normal average half-disappearance times of 18, 81, and 578 minutes, respectively. From the computer-analyzed data the normal bilirubin production rate in 13 normal subjects was

calculated to be  $3.8 \pm 0.6$  (SD) mg/kg/day.<sup>155</sup> Generally higher rates of bilirubin production were observed in hemolytic states and the data were expressed in terms of a calculated erythrocyte life span.<sup>155</sup> The results correlated well with other means of measuring erythrocyte life span.

**QUANTITATIVE EXCRETION OF UROBILINOGEN.** Quantitative fecal urobilinogen methods have been in use for some time and are employed in a number of clinical laboratories. They entail the timed collection of fecal specimens, usually over a four-day period. The average per diem excretion is determined from analysis of these specimens by methods based on the Ehrlich reaction<sup>199</sup> (page 215). Obviously, inaccuracies may be introduced by incomplete or inaccurately timed collections. Even if collection is adequate, there are reasons for considering urobilinogen excretion to be an imperfect index of heme breakdown (page 214).

The normal value for urobilinogen excretion is 40 to 280 mg/day.<sup>199</sup> However, this value is often expressed and better understood in relation to the estimated circulating hemoglobin mass<sup>182</sup> (page 727). Expressed in this relation, normal values of 11 to 21 mg per 100 g circulating hemoglobin have been observed; in hemolytic disease, values may be two to ten times greater.

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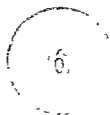
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## *Granulocytes and Monocytes*

### **Morphology and Chemical Properties**

#### **The Myeloid Series**

#### **The Monocyte Series**

#### **Differential Cell Counting**

### **Leukocyte Kinetics (Dynamics of Production, Circulation, and Turnover) and Cell Function**

### **Neutrophil Series—Kinetics, Properties, and Functions**

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#### **Phagocytosis and Particle Ingestion**

#### **Bacterial Killing and Digestion**

#### **Secretory Functions of the Neutrophil**

### **Eosinophil Series—Kinetics, Properties, and Functions**

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#### **Site of Production and Kinetics**

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#### **Properties and Functions of Monocytes and Phagocytes**

## **Morphology and Chemical Properties**

Evidence for the replenishment of marrow and blood cells from a stem cell compartment was presented in Chapter 2. The multipotential, colony-forming unit cell (CFU or uncommitted stem cell) and the intervening ("committed") stem cells for each cell line have not been identified morphologically by traditional methods, and reliable electron microscopic criteria for distinguishing myeloblasts from pronormoblasts or lymphoblasts also are lacking.<sup>57,62,68,73</sup> Only the more mature forms of each hematopoietic cell series can be reliably distinguished from one another. In the following pages these cells will be described and their properties and functions will be considered.

### **The Myeloid Series**

Neutrophilic, eosinophilic, and basophilic granulocytes comprise the myeloid series. All three cell lines are thought to follow similar patterns of proliferation, differentiation, maturation and storage in the bone marrow, and delivery to the blood. The details of these processes are best documented for neutrophils and are meager for basophils. The first three morphologic stages, the myeloblast, promyelocyte, and myelocyte, are capable of replication as is shown by their uptake of tritiated thymidine and the presence of mito-

ses; the later stages cannot divide but undergo differentiation and maturational changes. The morphologic boundaries of each cell compartment were defined many years ago and were based on criteria such as cell size, ratio of size of nucleus to cytoplasm, fineness of nuclear chromatin, nuclear shape, the presence or absence of nucleoli, the presence and type of cytoplasmic granules, and the cytoplasmic color of stained cells (Table 6-1, page 238).

Since changes in nuclear chromatin and cell size occur during each cell replication cycle (see Chapter 2) and since the formation of granules and other cytoplasmic changes occurs gradually during the stages of cell development, morphologic definitions are necessarily arbitrary and do not always conform to significant biochemical or physiologic changes. At times it is difficult to classify a cell in one category or another since it is actually in transition between the two. Nevertheless it is useful to separate the cell lines into morphologic compartments and to define normal limits of cell distribution therein since gross changes from these patterns are indicative of disease.

### *The Myeloblast*

The term "myeloblast" describes an immature cell, normally found in the bone marrow and not in the blood. This cell can divide and give rise to promyelocytes which in turn give rise to myelocytes. It is not known whether there are separate myeloblasts for the neutrophilic, eosinophilic, and basophilic cell lines, but, if there are, no reliable criteria for identifying them exist even at the electron microscopic level.<sup>73</sup>

The myeloblast (Plate V, A and Fig. 6-1, 1 to 4) possesses a relatively large nucleus, round or slightly oval in shape, and a small amount of cytoplasm. In preparations treated with Wright's stain, the nuclear membrane is smooth and even in outline and exceedingly thin, with no condensation of chromatin near its inner surface as in lymphoblasts. The chromatin shows an even, diffuse distribution

with no aggregation into larger masses, although there may be some condensation about the nucleoli. The chromatin may appear in the form of very fine strands, thus giving the nucleus a sieve-like appearance; or it may have the form of fine dust-like granules, producing a uniform stippled effect. There are, generally, from two to five nucleoli, pale sky-blue in color. It has been claimed that a large number of nucleoli favors the myeloblast as against the lymphoblast, but this has been disputed. The cytoplasm is basophilic (blue) and generally, although not invariably, there is no clear zone about the nucleus. Sometimes the cytoplasm is reticular, spongy, or foamy.

In electron microscopic sections the above observations in general are confirmed.<sup>17</sup> The nuclear membrane is thin and indistinct with little or no chromatin condensation. The numerous particles of ribonucleoprotein in the cytoplasm produce deep-blue basophilia in stained preparations. Mitochondria are abundant but small, and the endoplasmic reticulum is flat and appears infrequently. The Golgi apparatus is indistinct and no cytoplasmic granules are present.

Some authors classify what may be slightly more mature cells with several, rather large, angular, irregular and dark-staining azurophilic cytoplasmic granules as myeloblasts. It is simpler, however, to include such forms in the promyelocyte stage thus making the separation between the two cell types clear-cut. The electron microscopic classification of myeloid cells, which is based primarily on stages of granule formation, also places cells with beginning granule formation in the promyelocyte category.<sup>13,73</sup>

In wet films, myeloblasts appear immobile, with thin, tenuous borders. The cytoplasm is hazy and usually contains no stainable substance except mitochondria, which are diffusely scattered throughout the cytoplasm and stain brilliant blue-green with Janus green. The failure of myeloblasts to move in supravitral preparations is probably due to the nature of the preparation itself rather than to the cells' immobility. In motion picture studies of hanging-drop preparations, myeloblasts

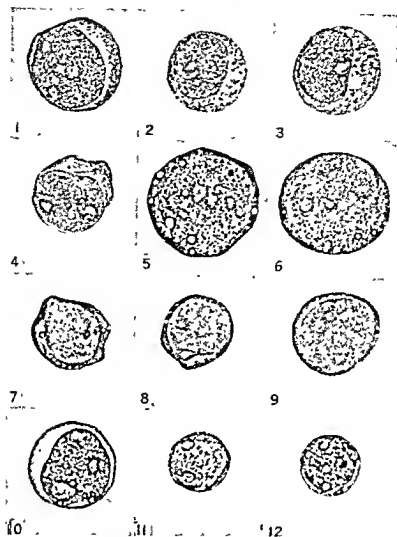


Fig. 6-1 Myeloblasts, myelocytes and lymphoblasts 1 2 3 and 4 Myeloblasts from patients with myeloblastic leukemia. In 4 there is an Auer body. 5 and 6 Promyelocytes from human marrow. 7 and 8 Very immature lymphoid cells (lymphoblasts) from a lymph node of a newly born rabbit. 9, 10 11 and 12, Lymphoblasts from patients with acute lymphoblastic leukemia (From Downey<sup>24</sup> courtesy of the author and Paul B. Hoeber.)

manifest a characteristic, snail-like movement.<sup>61</sup> The suggestion that this distinguishes them from lymphoblasts (Fig. 6-2) has been questioned, the different types of motility being ascribed to external factors.<sup>23</sup>

Since they are in the process of growth and division, myeloblasts vary considerably in size, from 10 to 20  $\mu\text{m}$  in diameter. Particularly in acute leukemia, the nucleus may show several wide and deep indentations suggesting lobulation. Such myeloblasts (see *Rieder cells*,<sup>24</sup> below) suggest a more rapid maturation on the part of the nucleus as compared

with the cytoplasm (asynchronism of DiGuglielmo).

Also in leukemia, *Auer bodies* are seen in the cytoplasm of cells which otherwise look like myeloblasts (Fig. 6-1).<sup>73</sup> Typical Auer bodies are rod-shaped cytoplasmic inclusions which stain with azure dyes and contain acid phosphatase,<sup>30</sup> peroxidase,<sup>32</sup> and esterase.<sup>35</sup> Some have considered them to be abnormal derivatives of primary granules,<sup>78</sup> and, as such, to signify beginning differentiation. However, there are differences between the fine structure of Auer rods



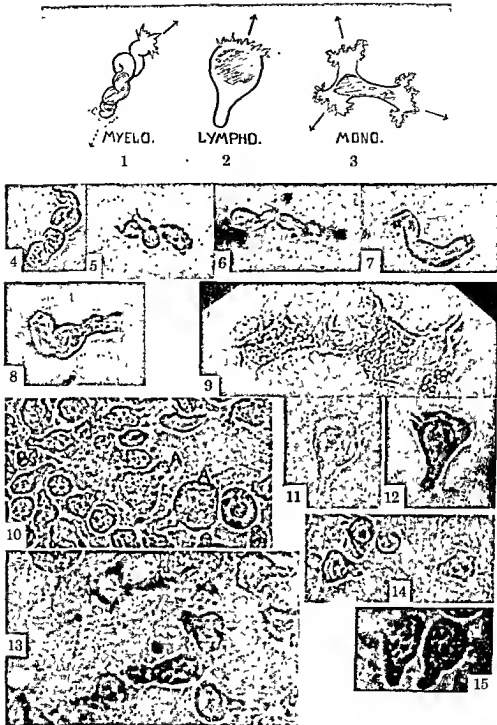


Fig 6-2. Diagrams (1 2 3) and photographs (4-15) of living moving unstained cells enlarged ( $\times 1000$ ) from negatives of motion picture films of tissue cultures. 1, "Worm-like" shape of myeloblast in motion. 2, Hand-mirror shape of lymphoblast in motion. 3, Shape of monocyte (histiocyte) in motion. 4 and 5, Myeloblasts from the bone marrow of a normal rabbit. 6, Myeloblast from the bone marrow of a normal person. 7 and 8, Myeloblasts from the blood of a patient with acute myeloblastic leukemia. 9, Two monocytes from normal blood. Note the broad, ruffled pseudopodia. 10, Cells from the lymph node of a three-week-old normal rabbit. There are two large, hand-mirror shaped, moving lymphoblasts (A); also note the "hand mirror" shape of moving small lymphocytes (B). 11, 12, and 13, Lymphoblasts from the blood of a patient with acute lymphoblastic leukemia. 14, Typical small lymphocytes from the blood of a patient with chronic lymphocytic leukemia. 15, Typical large lymphoid cells from the blood of a patient with infectious mononucleosis (Rich et al.<sup>41</sup> courtesy of the authors and Bulletin of Johns Hopkins Hospital.)

and primary granules.<sup>74,75</sup> Furthermore, Auer rods have been reported in monocytes, the characteristic granules of which differ substantially from those found in neutrophils.<sup>51</sup>

Rieder cells are not a specific cell type but probably represent asynchrony of nuclear and cytoplasmic differentiation in monocytes, lymphocytes, myeloblasts, leukoblasts, or reticuloendothelial monocytoïd cells; i.e., cells in which the nucleus is polymorphous or highly differentiated while the cytoplasm is immature.<sup>36</sup>

### Differentiation of Myeloblasts from Lymphoblasts and Other "Blasts"

It is extremely difficult, by present-day methods at least, to distinguish leukoblasts (myeloblasts, lymphoblasts, monoblasts) and even pronormoblasts if cells showing beginning maturational changes (granule formation or hemoglobin synthesis) are excluded from the blast category.<sup>73</sup> In the *lymphoblast* the nuclear membrane is denser than that of the myeloblast and the chromatin is coarser and may show some aggregation. These cells are contrasted in Plate XIX, A-F. There generally are only one or two nucleoli in lymphoblasts and their membrane usually is very distinct. The mitochondria are short and more plump than those of myeloblasts and often assume a position close to the nucleus. The "*monoblast*" is described as showing characteristics similar to those of the mature monocyte, such as very fine chromatin, pale nucleus, and ground-glass cytoplasm with a fine, irregular border. Additional morphologic differences will be given later in this chapter (page 234). In many instances, the identification of "blast" cells is greatly aided by the company they keep—the more mature and more easily recognized cells about them in sections, or in the same blood smear. In the case of the myeloblast, the demonstration of associated promyelocytes which show azure granulation in Wright's or similar stains, or positive reaction in the peroxidase stain, is strong presumptive evidence for this cell's identification.

### Myelocytes and Promyelocytes

The developmental stages in the granulocyte series and some of their morphologic variations are shown in Plate V, A-L, and Figure 6-3. Classically the several stages beyond the myeloblast have been differentiated primarily on the basis of the number and type of granules present, as in the A, B, and C myelocytes of Sabin<sup>64</sup> or the *promyelocyte* and differentiated *myelocyte* of Pappenheim. In these classical schemes it was generally assumed that there is an azurophilic, granule-containing cell, the progranulocyte or promyelocyte, which is a common precursor for the three granulocyte series,<sup>17</sup> and that during development these nonspecific granules degenerate and/or transform into the "specific" granules which characterize the mature neutrophil, eosinophil, or basophil. However, electron microscopic, histochemical, and biochemical evidence indicates that each granule population associated with the several stages of the myeloid series can be identified and related to a given cell line. The so-called "nonspecific, azurophilic," or *primary* granules first appear at the promyelocyte stage and can be identified on fine structural study as characteristic of the neutrophil, eosinophil, or basophil series.<sup>3,13,65,73</sup> They do not transform into "specific" granules but persist throughout the remainder of the maturation sequence and are seen in all subsequent stages including the polymorphonuclear forms<sup>10,13,65</sup> (Fig. 6-3).

The *promyelocyte* is somewhat larger on the average than the myeloblast. In both light and electron microscopic preparations it possesses a round or oval nucleus in which the nuclear chromatin is diffusely distributed, as in the myeloblast; in later stages, slight chromatin condensation is discerned around the nuclear membrane. Nucleoli are present, but as the cell develops they become less prominent. As compared with the myeloblast, the main difference, as seen with the electron microscope, is in the cytoplasm where the endoplasmic reticulum is more prominent and takes on a dilated, vesicular appearance. The azurophilic, primary granules appear and

Table 6-1 Morphologic Characteristics of the Leukocytes (Wright's Stain)

Type of Cell	Nucleus							Cytoplasm			
	Size	Position	Shape	Color	Chromatin	Nuclear Membrane	Nucleoli	Relative Amount	Color	Pen nuclear Clear Zone	Granules
1 Granulocytes											
(a) Myeloblast	10-18 $\mu$ m	Eccentric or central	Round or oval	Light reddish purple	Very fine meshwork	Very fine	2-5	Scanty	Blue	None	None
(b) Promyelocyte	12-20 $\mu$ m	Eccentric or central	Round or oval	Light reddish-purple	Very fine meshwork	Fine	2-5	Moderate	Blue	None	Azurophilic
(c) Myelocyte	12-18 $\mu$ m	Eccentric	Oval or slightly indented	Reddish-purple	Fine but becomes gradually coarser	Indistinct	Rare	Moderate	Bluish-pink	None	Azurophilic plus specific granules
(d) Metamyelocyte	10-18 $\mu$ m	Central or eccentric	Thick horseshoe or indented	Light purplish-blue	Basal and oxychromatin clearly distinguished	Present	None	Plentiful	Pink	None	Neutrophilic, eosinophilic, or basophilic
(e) "Juvenile" or band form	10-16 $\mu$ m	Central or eccentric	Band shape of uniform thickness	Light purplish-blue	Basal and oxychromatin clearly distinguished	Present	None	Plentiful	Pink	None	Neutrophilic, eosinophilic, or basophilic
(f) Polymorphonuclear neutrophil	10-15 $\mu$ m	Central or eccentric	2-5 or more distinct lobes	Deep purplish-blue	Rather coarse	Present	None	Plentiful	Faint pink	None	Fine, pink or violet pink
(g) Polymorphonuclear eosinophil	10-15 $\mu$ m	Central or eccentric	2-3 lobes	Purplish-blue	Coarse	Present	None	Plentiful	Pink	None	Large, coarse, uniform in size, crimson-red, numerous
(h) Polymorphonuclear basophil	10-15 $\mu$ m	Central	2-3 lobes	Purplish-blue	Coarse, overlaid with granules	Present	None	Plentiful	Faint pink	None	Large, coarse, uniform, bluish black

2. Lymphocytes	10-18 $\mu$ m	Eccentric or central	Round or oval	Light reddish-purple	Moderately coarse particles, "supplied"	Fairly dense	1-2	Scanty	Clear blue	Present	None
(a) Lymphoblasts											
(b) "Mature" lymphocyte	7-18 $\mu$ m	Eccentric	Round or slightly indented	Deep purplish-blue	Large masses of moderate or large size, or pyknotic	Dense	None	Scanty or plentiful	Sky-blue, deep blue, or even very pale pink	Present if cytoplasm is dark	None or few, azurophilic
3 Monocyte	12-20 $\mu$ m	Eccentric or central	Round, oval, notched or horseshoe	Pale bluish-violet	Fine reticulated, skein-like or lacy	Present	None	Abundant	Grayish or cloudy blue	None	Abundant, fine, lilac or reddish blue
Macrophage	15-80 $\mu$ m	Central	Elongated, indented or oval	Pale bluish-violet	Spongy	Distinct	None	Usually abundant	Opaque sky-blue	None	Numerous, moderately coarse azure granules and vacuoles

**Table 6-2. Morphologic Characteristics of the Leukocytes (Supravital Stain)**

<i>Type of Cell</i>	<i>Size, (micrometers)</i>	<i>Contour</i>	<i>Type of Motility</i>	<i>Nucleus</i>		
				<i>Shape</i>	<i>Chromatin</i>	<i>Nucleoli</i>
<b>1 Myeloid series</b>						
(a) Myeloblast	14-20	Smooth and sharp	None*	Round or oval	Loose meshwork	2-5
(b) Myelocyte	16-24	Distinct, smooth	None*	Round or oval	Gradually denser	Gradually disappear
(c) Metamyelocyte	12-18	Irregular	Slightly amoeboid	Reniform or horseshoe	Moderately dense	None
(d) Polymorphonuclear neutrophil	12-15	Irregular Repeated pseudopod formation	Actively amoeboid	Lobulated Orragged behind except in basophil	Coarse	None
(e) Eosinophil			Less active			
(f) Basophil			Active			
<b>2 Lymphoid series</b>						
(a) Lymphoblast	10-20	Sharp	None*	Round or oval	Sparse	1-2
(b) Young lymphocyte	9-18	Sharp	Nucleus at front static constriction rings	Round or indented	Slight	0-1
(c) Mature lymphocyte	7-18	Sharp	Nucleus at front static constriction rings	Round or indented	Moderate	0
(d) Old lymphocyte	7-15	Sharp	Nucleus at front static constriction rings	Usually round	Coarse	0
<b>3 Monocyte series</b>						
(a) Monoblast	12-18	Sharp or slightly irregular	None*	Round or oval	Very sparse	1-2
(b) Young monocyte	12-18	Irregular and lace like	Bleb-like pseudopodia surface film type	Indented	Slight	0-1
(c) Mature monocyte	16-20	Irregular and lace like	Bleb-like pseudopodia surface film type	Markedly indented	Moderate loose mesh	0
(d) Clasmato-cyte	15-80	Irregular	Bleb-like pseudopodia, surface film type	Elongated, indented or oval	Moderate	0-1

\*See text

Table 6-2. Continued.

Cytoplasm						
Relative Amount	Color	Neutral Red Vacuoles	Mitochondria		Physi- cal State	Evidence of Phago- cytosis
			Shape or Size	Distri- bution		
Scanty	Slightly yellow	None	Spherical	Numerous diffuse	Gel	None
Moderate	Gray	Refractile bright red A = 3-10 B = 30-100 C = full amt Beginning streaming	Spherical	Diffuse few in C type	Gel sol in C type	None
Plentiful	Gray	Beginning streaming Yellowish pink tiny refractile	Spherical	Few	Sol	Slight to moderate For bac- teria debris etc
Plentiful Clear ecto- and endo- plasm ex- cept in basophil	Homo- geneous and clear	Bright yellow large oval refractile Deep maroon non refractile all streaming	Spherical	Few	Sol	Slight  Slight
Scanty	Yellow	None	Short plump rods	Numerous esp close to nucleus	Gel	None
Mora	Grayish yellow	None or many Any distribution	Short plump rods	Numerous esp at one side of N	Sol	None
Usually plentiful	Gray	None or many Any distribution	Short plump rods	10-20	Sol	None
Usually scanty	Colorless	None or many Any distribution	Smaller	0-10	Sol	None
Scanty	Slightly yellow and "muddy"	None or vary fine in centro- spheres	Fine spheres or slender rods	Numerous	Gel	None
Moderate	Colorless and "muddy"	Numerous small	Fine dust like	Moderate number	Sol	Uncommon
Abundant	Colorless and "muddy"	Very numerous vary in size occas rosette	Fine dust- like	Few	Sol	Moderate
Usually abundant	Colorless	Often many but vary in number size and color	Delicate filaments	Few or none	Sol	Usually for particles or whole cells

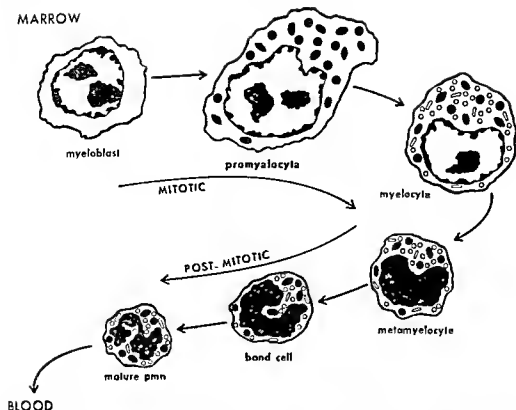


Fig 6-3. Diagrammatic representation of the appearance of granules during neutrophil maturation. Myeloblasts are relatively undifferentiated cells with a large oval nucleus, large nucleoli, and cytoplasm lacking granules. They originate from a precursor pool of stem cells. Subsequently, there are two secretory stages—the promyelocyla and the myelocyla—each of which produces a distinct type of secretory granule, azurophilic (dark granules) are produced only during the promyelocyla stage, specific granules (light granules) are produced during the myelocyla stage. The metamyelocyla and band forms are nonproliferating, nonsecretory stages that develop into the mature PMN characterized by a multilobulated nucleus and cytoplasm containing primarily glycogen and granules. Both nonspecific azurophilic granules and specific granules persist throughout these later stages. (Modified from Bainton et al.<sup>13</sup> courtesy of the author and the Journal of Experimental Medicine)

Fig 6-4. Early and late promyelocytes, a myelocyte, and a polymorphonuclear neutrophil viewed by electron microscopy (Courtesy D Bainton, University of California, San Francisco)

**A. Early neutrophilic promyelocyte** (reacted for peroxidase,  $\times 10,500$ ). The nucleus (N) with its prominent nucleolus (Nu) occupies the bulk of this very immature cell. The surrounding cytoplasm contains a few azurophilic granules (ag), a large Golgi complex (G), Golgi cisternae (Gc), several mitochondria (m), scanty rough endoplasmic reticulum (er), and many free polysomes (r). A centriole (cc) is present in the Golgi region.

All of the azurophilic granules (ag) appear dense, since they are strongly reactive for peroxidase. The secretory apparatus (ie, the perinuclear cisterna [pn], rough endoplasmic reticulum [er], and Golgi cisternae [Gc]) are also reactive, although less so than the granules.

Specimen fixed in glutaraldehyde for 16 hours at  $4^{\circ}\text{C}$ , incubated in the peroxidase medium of Graham and Karnovsky for one hour at  $22^{\circ}\text{C}$ , post fixed in  $\text{OsO}_4$ , treated in block with uranyl acetate, dehydrated in ethanol, infiltrated with propylene oxide, and embedded in Araldite. Section stained for one minute with lead citrate.

**B. Late neutrophilic promyelocyte** (reacted for peroxidase,  $\times 7,000$ ). This cell is the largest ( $\sim 15 \mu\text{m}$ ) of the neutrophilic series. It has a sizable, slightly indented nucleus (N), a prominent Golgi region (G) and cytoplasm packed with peroxidase-positive azurophilic granules (ag). Note the two general shapes of the azurophilic granules, spherical (ag) and ellipsoid (ag'). The majority are spherical, with a homogeneous matrix, but a few ellipsoid forms containing crystalloids also are present. Many of the spherical forms (ag) have a dense periphery and a lighter core, owing presumably to incomplete penetration of substrate into the compact centers of mature granules.

Peroxidase reaction product is visible (under higher magnification) in less concentrated form within all compartments of the secretory apparatus (endoplasmic reticulum, perinuclear cisterna, and Golgi cisternae). No reaction product is seen in the cytoplasmic matrix, mitochondria, or nucleus.

Specimen fixed in glutaraldehyde for 10 minutes at  $4^{\circ}\text{C}$  and subsequently processed exactly as was specimen A.



Fig. 6-4. (continued from page 23D)

C. *Neutrophilic myelocyte* (reacted for peroxidase  $\times 9\ 000$ ) At this stage the cell is smaller ( $\sim 10\ \mu\text{m}$ ) than the promyelocyte, the nucleus is more indented, and the cytoplasm contains two different types of granules: (1) large, peroxidase-positive azurophilic (ag) and (2) the generally smaller specific granules (sg), which do not stain for peroxidase. A number of immature specific granules (is), which are larger, less compact, and more irregular in contour than mature granules, are seen in the Golgi region (G). Note that peroxidase reaction product is present only in azurophilic granules, and is not seen in the rough endoplasmic reticulum (er), perinuclear cisterna (pn), and Golgi cisternae (Gc). This is in keeping with the fact that azurophil production has ceased and only peroxidase-negative specific granules are produced during the myelocyte stage.

D. *Mature PMN* (reacted for peroxidase,  $\times 10\ 500$ ) The cytoplasm is filled with granules, the smaller peroxidase-negative specific granules (sg) are more numerous, the azurophilic (ag) having been reduced in number by cell divisions after the promyelocyte stage. Some small, irregularly shaped azurophilic granule variants are also present (unlabeled arrow). The nucleus is condensed and lobulated ( $N^1-N^4$ ). The Golgi region (G) is small and lacks forming granules, the endoplasmic reticulum (er) is scanty, and mitochondria (m) are few. Note that the cytoplasm of this cell has a rather ragged, moth-eaten appearance due to the fact that the glycogen, which is normally present, has been extracted in this preparation by staining in block with uranyl acetate.



accumulate in increasing numbers during this stage, but the so-called specific or secondary granules are not yet present<sup>3,10,13,17,73</sup> (Fig. 6-4). In early promyelocytes the few granules present may be difficult to see by light microscopy as they often lie over the nucleus and are evident only on examination at several focal planes.

Like the myeloblast, the promyelocyte is immobile in flat slide and coverslip preparations and only in the last stage is slight locomotion seen. Even then the streaming of granules so characteristic of mature granulocytes is lacking.<sup>4</sup> For this reason the cytoplasm has been thought to be in the form of a gel; the increased resistance of the cytoplasm of immature myeloid cells to changes in shape has been proposed as a factor in retention of these cells in the bone marrow.<sup>16,17</sup> In hanging-drop preparations, promyelocytes are actively mobile, however.

The *neutrophilic myelocyte* may be defined as the stage in which *specific (secondary) granules* appear in the cytoplasm and the cell consequently can be identified as belonging to the neutrophilic series when stained and observed with the light microscope. As mentioned above, earlier identification of a cell that will become neutrophilic can be made by electron microscopic examination of the primary, "nonspecific" granules. The nucleus of the neutrophilic myelocyte usually is eccentric, round or oval in shape, and one side may appear somewhat flattened. The nuclear chromatin is somewhat coarse, and nucleoli are small and often not visible, although they are clearly seen with the electron microscope.<sup>3</sup> Primary granules persist in myelocytes, but formation of new primary granules stops abruptly, and each succeeding cell division leads to a decrease in their number (Fig. 6-3) in the daughter population.<sup>4,10,13</sup> The secondary granules of the neutrophil series are smaller than the primary granules and in the rabbit, cat, and man are formed in increasing numbers on the convex surface and lateral borders of the somewhat less prominent Golgi apparatus.<sup>3,10,13</sup> The amount of granular endoplasmic reticulum is reduced in the myelocyte as compared to earlier forms,

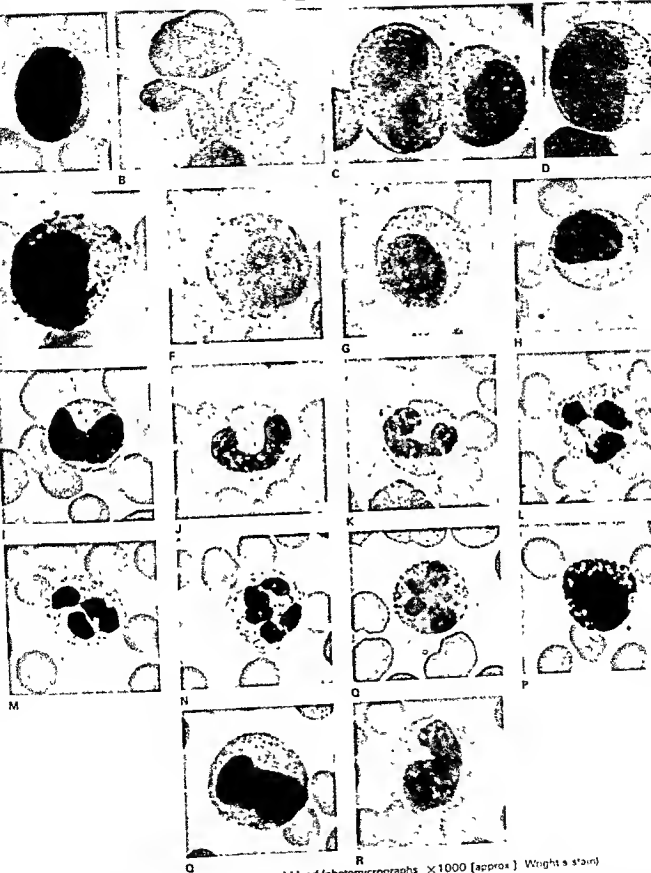
and thus the cytoplasmic basophilia decreases and disappears. The mitochondria remain small and relatively few in number.

### *Nature of Primary and Secondary ("Specific") Granules*

It has been suggested, from studies in the rabbit and cat, that the primary granules are packaged and released from the inner, concave surface of the Golgi apparatus (Fig. 6-5A) in contrast to the secondary (so-called "specific") granules of the myelocyte and later granulocyte stages which appear to be formed and released from the outer, convex surface.<sup>3,10,13,65</sup> Recently this also has been shown to be the case in man.<sup>13</sup> Some workers, however, believe that the primary granules are formed diffusely throughout the cytoplasm by the condensation of precursor vacuoles without the Golgi apparatus being involved in their formation.<sup>65</sup> In the mature neutrophil, a ratio of secondary to primary granules of about 9 to 1 is seen in the rabbit<sup>73</sup> and 2 to 1 in man.<sup>13</sup>

The mature *primary granules of human neutrophils* usually contain central crystalloids when lightly stained.<sup>65</sup> They apparently bind neutral red dye and thus are easily seen as neutral red bodies in supravital preparations.<sup>2</sup> They are membrane-bound lysosomes which contain a number of enzymes and other substances, such as acid phosphatase, peroxidase, esterase,  $\beta$ -glucuronidase,  $\beta$ -galactosidase, aryl-sulfatase, 5'-nucleosidase, N-acetyl- $\beta$ -glucosaminidase,  $\alpha$ -mannosidase, sulfated mucosubstance, lysozyme and other basic proteins.<sup>13,73</sup> There is considerable variation in *acid phosphatase* activity as reported in different studies and in different species.<sup>3,10,11</sup> This may be due to inadequacies of the histochemical assays or perhaps is related to species variations.<sup>73</sup> *Peroxidase* has been associated with primary granules by histochemical, cytochemical, and biochemical methods and at the present time is the best marker for primary granules in mammals.<sup>73</sup> *Sulfated mucosubstance* presumably accounts for the azurophilic staining of the primary granules; the uptake of radiosulfate by early

# PLATE V



Normal leukocytes from bone marrow and blood (photomicrographs  $\times 1000$  [approx.] Wright's stain)

A Myeloblast, B. Myeloblast with myelocyte and late metamyelocyte C Two promyelocytes D Promyelocyte  
 E. Late promyelocyte or myelocyte F. Myelocyte G. Myelocyte H. Late myelocyte or early metamyelocyte I  
 Metamyelocyte, J, Band neutrophil, K, Band neutrophil, L, Polymorphonuclear neutrophil M, Polymorphonuclear  
 neutrophil N, Eosinophil, P, Basophil Q Monocyte R Monocyte

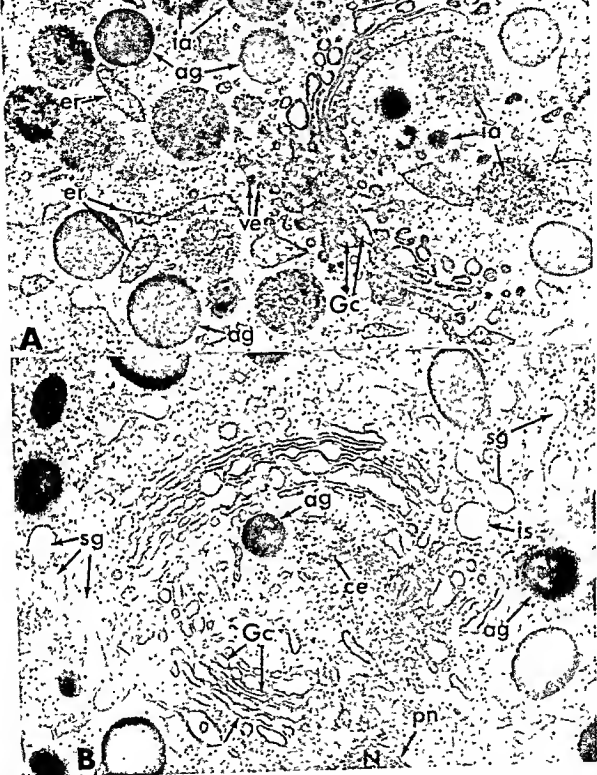


Fig 6-5. Granule formation in neutrophil precursors viewed by electron microscopy (Courtesy D. Bainton, University of California, San Francisco)

A. Golgi region of a neutrophilic promyelocyte reacted for peroxidase ( $\times 40,000$ ). At this stage the peroxidase reaction product is present within the rough endoplasmic reticulum (er), the clusters of smooth vesicles (vel) located at the periphery of the Golgi cisternae (Gc) in the Golgi cisternae, and in the immature (ia) and mature (ag) azurophilic granules. The immature granules are larger and less compact than the uniformly dense mature granules.

B. Golgi region of a neutrophilic myelocyte reacted for peroxidase ( $\times 40,000$ ). Peroxidase reactive material is seen in the primary or azurophilic granules (sg) but not in the specific (secondary) granules (ag). At this stage (myelocyte) no peroxidase reaction product is seen in the endoplasmic reticulum, Golgi cisternae (Gc) or newly formed immature specific granules (is). The stacked Golgi cisternae are oriented around the centriole (ce) and the outer cisternae (unlabeled arrow) contain material of intermediate density that is similar to the content of the specific granules, suggesting that the specific granules arise from the convex face of the Golgi complex as in the rabbit.

neutrophils<sup>43,70</sup> may be due to incorporation into this substance. About *one third* of neutrophil *lysozyme* appears to be present in the primary granules of rabbit neutrophils<sup>9</sup> and several other bactericidal, basic proteins have been localized in the primary granule fraction as well.<sup>79</sup> A number of *acid hydrolases* such as  $\beta$ -glucuronidase have been found in mixed granule fractions. *Alkaline phosphatase* appears to be absent from the primary granules of rabbits and man.<sup>11,13,73</sup>

The *secondary* granules of the neutrophil are characterized by their content of alkaline phosphatase and lack of acid phosphatase<sup>13,73,79</sup>; they also lack or contain very little peroxidase and sulfated mucosaccharide.<sup>73</sup> In addition, secondary granules contain amino peptidase, lysozyme, collagenase, and basic proteins. *Alkaline phosphatase* has been found in blood and exudate neutrophils by many workers, but considerable variation is seen between species<sup>3,11,73</sup> and in pathologic states.<sup>38</sup> Histochemical<sup>11,13,73,75</sup> and biochemical studies<sup>9,79</sup> indicate that this enzyme is located in the secondary granules of the granulocytes in man and in rabbits. *Lysozyme* has been identified in human neutrophil secondary granules<sup>31</sup> as well as in primary granules; about two thirds of this antibacterial basic protein is in the secondary granule.<sup>9,79</sup> *Aminopeptidase* appears relatively late in myelocyte maturation,<sup>2</sup> and *collagenase* has been reported in human blood granulocytes.<sup>41</sup> A variety of basic proteins also has been reported in cytoplasmic fractions in which the secondary granules predominate.<sup>73</sup>

A third form or *tertiary granule* has been described in human neutrophils,<sup>65,71</sup> but some investigators regard this as merely a morphologic variation of primary and secondary granules.<sup>13</sup> The "tertiary" granules first appear during the late myelocyte stage and are described in late marrow and blood forms. Whether these granules are formed beyond the myelocyte stage is unknown, but this has been thought to be likely.<sup>65</sup>

### *Metamyelocytes*

The metamyelocyte is characterized by a clearly indented or horseshoe-shaped nucleus

(Plate V, B, H, I) without nucleoli (even by EM examination) and the nuclear chromatin is moderately dense with considerable clumping evident along the nuclear membrane. The cytoplasm is filled with both primary and secondary (and, in man, perhaps tertiary<sup>65</sup>) granules, but the secondary granules predominate. The endoplasmic reticulum is sparse as are polysomes, thus signifying the virtual completion of protein synthesis.

The boundary between the myelocyte and metamyelocyte compartments is best defined physiologically by the fact that myelocytes synthesize DNA, take up tritiated thymidine into their nuclear chromatin, divide, and are actively involved in protein synthesis as evidenced by the presence of nucleoli, abundant endoplasmic reticulum, and polysomes. Before modern techniques became available, differentiation between myelocytes and metamyelocytes was defined mainly in terms of nuclear shape. This now is recognized as a poor criterion since it has been shown in time-lapse microcinematographic studies of human neutrophils that myelocyte nuclei may assume a markedly indented shape and may subsequently revert to an oval configuration and enter mitosis.<sup>14</sup> Consequently, in classifying cells at this stage, particular attention should be paid to evidence in the nucleus and cytoplasm that protein synthesis has decreased or stopped. This is judged by the fact that the nuclear chromatin is coarse and clumped and by the faint pink of the cytoplasm which in stained preparations is essentially the color of the mature cell. These features also are helpful in differentiating metamyelocytes (Plate V, H, 7) from monocytes (Plates V, Q, R and VI, 22-25) since, in the latter, nuclear chromatin remains fine and evidence of protein synthesis persists. Amoeboid movement is apparent in metamyelocytes, even in coverslip slide preparations.

### *"Juvenile" or "Band" Forms* (Plate V, J, K)

This stage is characterized by further condensation of nuclear chromatin and transformation of nuclear shapes into sausage or band configurations which possess approxi-

mately uniform diameters throughout their length (Fig. 6-3). Subsequently one or more constrictions begin to develop and progress until the nucleus is divided into two or more lobes connected by filamentous strands of heterochromatin, the polymorphonuclear stage. There has been considerable difference of opinion concerning the differentiation of the juvenile (band) and polymorphonuclear stages. Some workers require a clearly visible filamentous strand between lobes (Plate V, N) before classifying a cell as a polymorphonuclear form; anything less clear-cut, whether due to overlapping of nuclear lobes or to incomplete constriction, is classified as a band form.<sup>55,60</sup> Others regard a constriction greater than one half or two thirds of the nuclear breadth (Plate V, L) as adequate evidence of lobulation and classify such cells as polymorphonuclear,<sup>18,26</sup> or use other slightly different criteria.<sup>58</sup> Still others avoid the issue entirely. Unfortunately, no clear agreement has been reached. Since, as far as is known, there is no clear difference between band and segmented stages other than nuclear shape and a slightly earlier appearance of tritiated thymidine in the band forms, the distinction becomes an arbitrary one. Nevertheless, a clear and easily recognizable separation is needed if one wishes to count nuclear lobes for diagnostic purposes, as in the early detection of folic acid deficiency<sup>33</sup> or in assessing marrow release of young forms into the blood.<sup>50</sup> For such purposes we have chosen as the criterion for inclusion in the polymorphonuclear category the clear separation of nuclear lobes.<sup>55</sup> Cells without this complete formation of distinct lobes are classified as band forms.

#### *Polymorphonuclear Neutrophils* (Plate V, L, M, N)

In this stage of full maturity the cells usually are of essentially uniform size and granule content. In Wright-stained preparations the nucleus is found to take a deep, purplish color and contains coarse, condensed chromatin. The lobes are joined by thin filaments of chromatin although these may not be easily visible if the lobes are partially super-

imposed on one another. Careful examination by focusing through several planes may facilitate identification. The cytoplasm is faint pink and contains fine, specific granules which sometimes may give only a ground-glass appearance. The azurophilic, primary granules have usually lost their dark-staining characteristics by this stage, but can be seen with the electron microscope. Under the electron microscope the granules exhibit considerable variation in density, presumably a reflection of variation in enzyme content, and they maintain a minimum distance of 100 nm from the cell membrane.<sup>81</sup> Large masses of glycogen become evident for the first time in mature neutrophils; this may reflect their propensity for anaerobic metabolism. The purpose of nuclear lobulation is not known. Perhaps it enhances cell deformability and movement through vessel walls and into sites of inflammation, or perhaps nuclear segmentation results from nucleolar emptying and has no function.<sup>274</sup> Arneth believed that nuclear lobulation continues as the cell ages and that granulocytes with three or four lobes are more mature than those with only two.<sup>5</sup> This may be true in the sense that once nuclear constriction begins the completion of lobulation may continue for some time; during this interval the cell may be delivered to the blood and thus completion of lobulation may occur in the blood (or in cultures). However, the number of lobes a neutrophil will develop appears to be determined in the band stage (or earlier), and the time of appearance of neutrophils in the blood after pulse labeling with tritiated thymidine was found to be unrelated to the number of nuclear lobes.<sup>211</sup>

In wet films, marked amoeboid activity of polymorphonuclear neutrophils at physiologic temperatures is characteristic. First, long pseudopods of granule-free ectoplasm are extended and into this the endoplasm with its dancing granules advances. The cell moves in this fashion, generally, but erratically, in one direction for a considerable period of time. The nucleus follows passively and may be elongated or compressed into bizarre shapes. Often, as the cell proceeds, a loop strand of cytoplasm remains stretched out behind it. Finally this is drawn up or occa-

sionally it may be broken off. The rate of neutrophil locomotion has been estimated at 19  $\mu\text{m}$  per minute.<sup>45</sup> In supravitaly stained films the (specific) granules appear yellowish pink and are refractile; in addition, occasional larger, nonrefractile deep-red vacuoles may be seen.

"Senile" polymorphonuclear leukocytes that are no longer motile and fail to take up the neutral red stain have been described in *in vitro* preparations.<sup>64</sup> They are seen in small numbers in the blood where their survival time is short.<sup>212</sup>

### *Eosinophils* (Plate V, O)

Eosinophils exhibit the same maturation phenomena as neutrophils except that probably only one type of granule is formed.<sup>73</sup> In rabbits the earliest identifiable eosinophils have fine nuclear chromatin and well-developed nucleoli, and contain large mitochondria, dilated endoplasmic reticulum, and a few large, dense, homogenous granules. These homogenous granules appear to be formed throughout all the subsequent stages of maturation. Their contents are first seen as flocculent material in Golgi saccules, then in small vacuoles which condense to form the large homogenous, dense granules. Subsequently, crystalloids develop and the granules acquire an angular shape; the angular configuration predominates in the mature polymorphonuclear forms and is also seen in human eosinophils.<sup>71</sup> Condensation in nuclear chromatin, decrease in size and ultimate disappearance of nucleoli, and reduction in the size of the Golgi apparatus parallel the changes seen during neutrophil maturation (Fig. 6-3). It is unusual to find more than two lobes in mature eosinophils and the lobes are larger than those seen in neutrophils. Eosinophilic granules are considerably larger than neutrophilic granules, appear somewhat refractile under the light microscope, and stain a bright yellowish red with Wright's stain.<sup>4</sup> Eosinophils are less actively motile than are neutrophils.

Cytochemically, eosinophilic granules (both homogenous and crystalline) are composed of a zinc-containing basic protein and peroxidase, but lack alkaline phosphatase, lysozyme, and bactericidal proteins. In addition, a number of other components have been identified in the granules: PAS reactive mucosubstance, acid phosphatase, ribonuclease,  $\beta$ -glucuronidase, cathepsin, aryl sulfatase, deoxyribonuclease, nucleotidase, lipase, and plasminogen.<sup>73</sup> Of the mature granulocytes, eosinophils display the most intense staining with peroxidase; the intense eosinophilic staining appears to be due to their basic protein content.

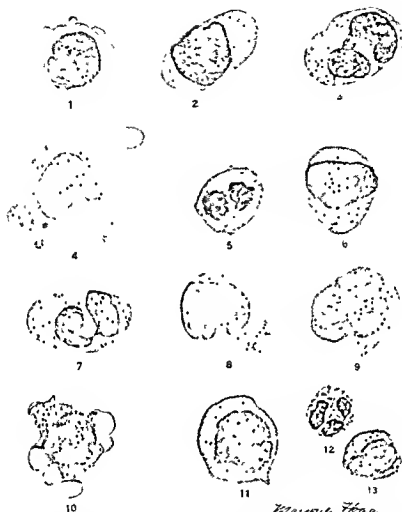
### *Basophils and "Mast" Cells* (Plate V, P)

Basophils are distinguished by their large, coarse, purplish-black granules (Plate V, P) that usually fill the cytoplasm and often obscure the nucleus. The granules are water soluble and thus may be dissolved in the process of staining and washing; the cells may then appear vacuolated with only a few or no basophilic granules remaining. On electron microscopic examination there is similar variation in the appearance of the basophilic granules. This is thought to reflect variable extraction of granule contents during preparative procedures rather than sequential production of several granule types as in the neutrophil.<sup>73,80</sup> In segmented basophils, mitochondria are large and numerous as in the eosinophil. In contrast to neutrophils, glycogen is much less abundant.<sup>71</sup>

Basophils exhibit sluggish motility; the nucleus advances rather than the cytoplasm as in the neutrophil and eosinophil.<sup>4</sup> The granules contain large amounts of heparin and histamine<sup>2,81</sup> (sulfated acid mucosubstance) and this is probably responsible for the affinity of the granules for basic dyes. Aryl sulfatase probably also is present, but peroxidase and alkaline and acid phosphatase are not.<sup>73</sup>

Similar but somewhat larger cells are found in the tissues and are known as *mast*

# PLATE VI



*Monocytes from the blood of a patient who died of miliary tuberculosis. Similar cells were seen in the tissues about the tuberculous nodules.*

11, Typical monocyte, 1, 2, and 6 have nuclei which are a little darker and are somewhat more stippled than is usual but are otherwise typical monocytes.

13, Small monocyte.

9, Most probably is a monocyte even though the nucleus is somewhat less distinct than in the other cells.

3 and 7, Heavily granulated monocytes with altered nucleus.

4, 8, and 10, Monocytes with histiocytic characteristics.

5, Cell in mitosis. The cytoplasm is basophilic. The cell may be lymphoid in origin.

12, Polymorphonuclear leukocyte showing "toxic" granulation.

cells. Although these cells resemble basophils in their metachromasia, acid nature, and content of histamine and heparin, they contain hydrolytic enzymes and 5-hydroxytryptamine<sup>1</sup> which basophils do not.<sup>12</sup> Also the ultrastructure of their granules is different in both man and the guinea pig.<sup>12</sup> Mast cells appear to discharge their granules outside the cell ("exoplasmosis"), while basophils usually undergo diffuse internal degranulation after phagocytosis although they may also exhibit exoplasmosis.<sup>81</sup>

### *Macropolycytes*

Macropolycyte is the name applied to giant polymorphonuclear neutrophils with a diameter greater than 16  $\mu\text{m}$  (as compared to normal mean 10 to 12  $\mu\text{m}$ , upper limits 15  $\mu\text{m}$ <sup>58</sup>) and possess from 6 to 14 nuclear lobes.<sup>39</sup> Such cells are seen only very occasionally in healthy subjects (1.3%), but they are found in about 5% of subjects with infections of various types or with intoxications, usually in association with a neutrophilic leukocytosis and the presence of myelocytes in the blood.<sup>39</sup> Macropolycytes are most commonly seen in association with folic acid or vitamin B<sub>12</sub> deficiency. We have often seen them also in patients recovering from the pancytopenia that attends treatment with cytotoxic agents.

Some authors have described cells with hypersegmented nuclei but of a normal size and refer to them as polycytes<sup>60</sup> or polylobocytes<sup>58</sup>; similar cells with complex nuclei but without hypersegmentation are called polypolycytes.<sup>60</sup> The latter forms are seen in perhaps 10% of patients recovering from leukocytosis with a marked shift to the left (see page 242) and appear in increasing numbers when anticoagulated blood is allowed to stand *in vitro*.<sup>56,60</sup>

The mechanism of macropolycyte formation is unknown, but it has been suggested that the skipping of one of the usual cell divisions that occurs during maturation results in a hypersegmented cell.<sup>72</sup>

### *Genetic Sex as Indicated by Leukocytes*

Only one X chromosome is essential to the normal activity of a cell; the other in the normal XX female remains unextended and thus is visible as a chromatin body. Sex chromatin (Barr) bodies are present in 80 to 90% of the somatic cells of the normal female. The sex chromatin body of the neutrophil of females is a small mass, usually adjacent to the nuclear membrane which stains deeply with hematoxylin, Feulgen reagent, and thionin and is about 0.7 to 1.2  $\mu\text{m}$  in size. It takes the form of a "drumstick" projecting from one of the nuclear lobes of about 2 to 3% (extreme range 1 to 17%) of the segmented neutrophils in the blood.<sup>21</sup> The "drumsticks" are well-defined, solid, round projections of chromatin connected to a lobe by a single fine chromatin strand (Fig. 6-6). They must be distinguished from "small clubbed" or racket-structured, nonspecific nodules which may be of smaller or larger size and are irregular in shape or lacking in chromatin, as well as from small (minor) lobes attached to the rest of the nucleus by two strands. Sessile nodules are equally sex specific but are more difficult to recognize.<sup>49</sup> Drumsticks are not found in normal males.<sup>21</sup>

It has been claimed that a sex chromatin body can also be identified in lymphocytes, monocytes, and nonsegmented neutrophils as a planoconvex or elliptical mass of dark-staining chromatin closely applied to the inner aspect of the nuclear membrane, but there is some doubt that this represents true sexual chromatin.<sup>52,53</sup>

The number of chromatin bodies seen in a cell is one less than the number of X chromosomes present. With the increased numbers of X chromosomes found in certain disorders of human development the number of Barr bodies and drumsticks increases and isochromosomes formed by duplication of the long arms of the X chromosome give rise to larger drumsticks than are found in the normal female.<sup>20</sup> Drumsticks or sessile nodules are seen in chromatin-positive males





Fig 6-6. Granulocytes, to show the sex chromatin. The characteristic drumsticks found in the female are shown in the two cells on the left, the thin strand of chromatin joining the head to a nuclear lobe can be seen clearly. In the two cells on the right "small clubs" are present such as may be seen in males. These should not be confused with drumsticks. (Wright's stain  $\times 1300$ .)

with Klinefelter's syndrome and are absent in chromatin-negative females with Turner's syndrome. Eosinophils and probably basophils also possess drumsticks. Drumsticks may be difficult to find in the presence of a marked shift to the left. Drumstick counts have been reported to be low in the leukocytes of patients with chronic myelocytic leukemia, paralleling the low leukocyte alkaline phosphatase and catalase concentrations.<sup>67</sup>

Double drumsticks<sup>49</sup> or a sessile nodule plus a drumstick in the same neutrophil is very rare.<sup>22</sup> Chromosomal abnormalities have been described in the leukocytes of  $D_1$  (13-15)-trisomy<sup>33</sup> and in mongolism (Down's syndrome).<sup>40</sup> Chromosome duplication has been studied in cultured human leukocytes.<sup>8</sup>

### The Monocyte Series

The monocyte is a phagocytic leukocyte found in the blood of vertebrates. So named by Pappenheim and Ferrata<sup>118</sup> the monocyte was first clearly differentiated by Schilling in 1912. It had been referred to as a "transitional cell" by Ehrlich because he thought it represented a transitional stage between immature mononuclear cells and the neutrophilic leukocyte.

Sabin and coworkers<sup>98</sup> regarded these cells as derived from a specific stem cell, the *monoblast*. The monoblast was described as being nonmotile, about  $14\ \mu\text{m}$  or greater in diameter, and characterized by basophilic or grayish cytoplasm, a large nucleus with little indentation, fine, stringy chromatin and one

or two large nucleoli. Numerous fine, spherical or slender, rod-like mitochondria appear in supravital stained cells, but neutral red vacuoles are absent or only a very few fine bodies are seen. These are presumably pinocytotic vesicles. On electron microscopic examination, aggregated ribosomes and scattered strips of endoplasmic reticulum can be seen in the cytoplasm, but the Golgi apparatus is small.<sup>105</sup> Differentiation of the monoblast from the myeloblast is difficult and probably impossible, even at the electron microscopic level.

### Promonocytes

The promonocyte or young monocyte is up to  $20\ \mu\text{m}$  in diameter and possesses a large, moderately indented nucleus. A nucleolus may be visible on light microscopy. The cell is motile and phagocytic but less so than the mature monocyte.<sup>110</sup> The cytoplasm shows considerable basophilia. No azurophilic granules are seen on light microscopy, but a few fine neutral red bodies may be distinguished. The cytoplasmic border often exhibits uneven extensions, or ruffled pseudopods. On electron microscopy, endoplasmic reticulum and ribosomes are evident and the Golgi apparatus is well developed with beginning cytoplasmic granule formation.<sup>113</sup>

### Monocytes (Plates V, Q, R, VI)

The mature monocyte is easily distinguished from other cells of the blood in well-stained preparations. It is usually larger

than other leukocytes (16 to 20  $\mu\text{m}$ ) and possess a large, oval or somewhat indented nucleus. The nuclear chromatin is delicate and the membrane is thin. The nucleus usually is centrally placed and one or two small nucleoli may rarely be present. The cytoplasm is abundant, grayish or muddy blue in color (Wright's stain), and is filled with myriads of fine lilac or reddish-blue vacuoles (Plate VI). If the preparation is not over-stained, the granules resemble fine dust or give the bluish cytoplasm a ground-glass appearance; if the stain is heavy, the granules appear more prominent and the cell may be confused with a metamyelocyte or even a band form of neutrophil. The very delicate chromatin and the bluish color of the cytoplasm in the monocyte are most helpful in differentiation. Granules in monocytes are stained by the peroxidase method but are fewer and finer than those in granulocytes.

In *supravital* stained films, numerous (40 to 80) neutral red bodies, varying in size from minute, dust-like granules to rather large vacuoles, are seen, often congregated near the nuclear hof. In some species (rabbit and guinea pig) these form a "rosette," but in man this arrangement is not usual.<sup>4,109</sup> Many spherical mitochondria can be seen scattered throughout the cytoplasm in preparations stained with Janus green.<sup>4</sup> The motility of the monocyte is characteristic in that large, filmy, irregular pseudopods extend slowly from the delicate cytoplasm and the cell advances in a sliding fashion with constant fluttering and waving of these projections (Fig. 6-2). On *electron microscopic examination* the mature monocyte contains a horseshoe-shaped nucleus, with dense, granular peripheral chromatin surrounding an extensive, light-staining central nucleoplasm. Nucleoli have been observed in as many as 50% of blood monocytes.<sup>113</sup> The mitochondria are round or elongated and are usually located in the periphery of the abundant cytoplasm. The Golgi apparatus is well developed and small vesicles are especially numerous in this region but may be found throughout the cytoplasm. On morphologic grounds these vesicles appear to be of several types.<sup>110,113</sup> In

addition, oval or rod-shaped dense bodies are seen in the perinuclear region and filaments have been described in the perinuclear and centriole regions<sup>110</sup> and throughout the cytoplasm.<sup>113</sup>

#### *Macrophage, Clasmatocyte, or Histiocyte*

Whether these cell forms are closely related or of very different origins was at one time debated.<sup>98,124</sup> Metchnikoff used the term "macrophage" (as opposed to microphage) to refer to all the mononuclear phagocytes found in tissues. Aschoff used the term "histiocyte" to include both *monocytes* and *clasmatocytes* which he regarded as identical. Sabin, on the other hand, although initially agreeing with this concept, subsequently concluded that there are two types of wandering phagocytes, the clasmatocyte and the monocyte. She regarded the Kupffer cells as anchored endothelial phagocytes with the same endothelial origin as the clasmatocyte; she believed that the monocyte was of different origin.

In 1939 Ebert and Florey showed that inflammatory macrophages are derived from blood monocytes,<sup>102</sup> supporting a view previously held by many workers.<sup>122</sup> Others argued that mononuclear phagocytes are derived from lymphocytes or from the reticulo-endothelial system.<sup>103,121</sup> In more recent studies in which allogeneic bone marrow cells or tritiated-thymidine-labeled syngeneic marrow cells were transfused into irradiated recipients,<sup>92,108,133</sup> the myelogenous origin of mononuclear phagocytes was clearly demonstrated (see page 267). Studies in splenectomized animals ruled out the spleen as a major source of macrophages.<sup>130</sup> In addition, lymphocytes isolated from blood did not become macrophages in cultures while monocytes did.<sup>119,129</sup> These findings and the time of appearance of tritiated thymidine in blood monocytes and peritoneal macrophages appear to have established the marrow as the source of blood monocytes and these cells in turn as the major source of macrophages in sites of inflammation (see page 267).<sup>129</sup>

The *macrophage* is a large, actively phagocytic cell (15 to 80  $\mu\text{m}$  in diameter) with an irregular shape. Its motility is similar to that of the monocyte. Macrophages having bleb-like and filiform pseudopodia are commonly seen. The cytoplasm is abundant and has many neutral red bodies scattered throughout it.<sup>90</sup> The nucleus is egg-shaped or may be indented or elongated. When stained with Wright's stain the chromatin appears "spongy" and the nuclear membrane is distinct. The cytoplasm is sky-blue and contains many coarse, azure granules and vacuoles. Electron microscopy of mouse peritoneal macrophages has demonstrated a spectrum of cell types ranging from a few small cells resembling blood monocytes to large ones with extensive cytoplasm, Golgi apparatus, and lysosomal granules.<sup>110</sup> A similar sequence of changes can be observed as human monocytes differentiate in tissue culture into macrophages, and subsequently into epithelioid cells and giant cells.<sup>107,122</sup>

The differentiation and maturation of mononuclear phagocytes have been studied under in vitro and in vivo conditions and the morphologic and biochemical events have been described in some detail.<sup>90,95,110</sup> In general, there is considerable variation depending on the site from which the macrophages are derived (peritoneum, pulmonary alveoli, sites of inflammation) and their prior exposure to conditions that induce or accelerate formation of hydrolytic enzymes and other manifestations of differentiation.<sup>90</sup> These morphologic variations presumably account for the different designations, such as clasmatocyte and histiocyte, used in earlier literature.<sup>98,99</sup>

### *Epithelioid Cells and Giant Cells*

Monocytes transform into macrophages which in turn may develop the characteristics of epithelioid cells, and then may ultimately fuse to form multinucleated giant cells. These changes have been followed in cultures of blood monocytes from humans<sup>107</sup> and other animals<sup>122,127</sup> and in inflammatory lesions such as experimental tuberculosis in rab-

bbits.<sup>100,125</sup> The epithelioid cell is larger than the monocyte and possesses an indented or oval nucleus with heavy chromatin. The cytoplasm is abundant and contains a great many vacuoles, a few mitochondria, and cytoplasmic filaments. The cell is highly phagocytic and intense protein synthesis is evident. It has been claimed that several types of giant cells are formed either by fusion of epithelioid cells or by amitotic division of monocytes,<sup>125</sup> but it seems likely that these several forms reflect minor variations of the same process. In giant cells, multiple Golgi regions are seen, mitochondria become prominent, and cytoplasmic filaments are quite numerous, whereas lysosomes become less prominent or absent, perhaps as a result of a change in function from one of phagocytosis and particle removal to one of active transport.<sup>127</sup>

*Lymphocytes* and plasma cells are described in Chapter 7.

The *morphologic characteristics of the various leukocytes* are summarized in Tables 6-1 and 6-2. Nucleated forms of the erythrocyte series are easily distinguished from most of the leukocytes by their lack of cytoplasmic granules. In Romanowsky-stained films, confusion arises only in the very immature (blast) stage. Even then the more granular and clumped nuclear chromatin may help to identify the pronormoblasts. Polychromatophilic normoblasts at times may be confused with plasma cells or lymphocytes. In the polychromatophilic normoblast the nucleus is more centrally placed than that of the plasma cell or lymphocyte, the cytoplasm is blue-pink in color, and the cell border may be irregular. The mature lymphocyte is characterized by coarse, clumped chromatin in a nucleus which is eccentrically placed in sparse cytoplasm; a few granules may be present also (Fig. 7-1). Deep blue cytoplasm in a cell with an eccentric nucleus containing very coarse, clumped chromatin is the hallmark of the plasma cell (Chapter 7).

The nucleated cells of the red cell series are nonmotile and in wet films have a rounded, distinct border with homogeneous, nongranular yellowish cytoplasm. The nucleus is round or oval and centrally placed.

The chromatin arrangement gives the nucleus a vesicular appearance and in early forms one or two large nucleoli are present. In supravitality stained preparations no neutral red bodies are seen, but many coarse, rod-shaped and coccoid mitochondria are found scattered diffusely in the cytoplasm.

The student will find it valuable, when learning to identify the various cells of the blood, to systematically seek each of the morphologic criteria listed in Tables 6-1 and 6-2. By doing so he will acquire the habit of seeing all that he is looking at, and in time he will learn to identify cells because of a number of characteristics which he perceives unconsciously. The actual identification of cells regarding which there may be some doubt can be made only by weighing the evidence for and against each type being considered. It must be kept in mind that practically no character of a cell is entirely specific. Thus, a perinuclear clear zone is sometimes seen in cells other than lymphocytes and a rosette of neutral red bodies has been observed in many types of blood and connective tissue cells other than monocytes.

### Differential Cell Counting

Differential cell counting refers to the enumeration and classification of the leukocytes seen on the blood smear. The usual procedure is to count at least 100 leukocytes while systematically scanning the smear in an area of good cell distribution. A uniformly thin smear of blood on a coverslip is the most satisfactory preparation for such examination (see Chapter 1, page 24). Smears drawn or pulled on slides are less suitable because the smear tends to be thicker and the larger leukocytes accumulate at the edges while the smaller ones, usually lymphocytes, become concentrated in the central portions of the film.<sup>154</sup> Ideally all the leukocytes on both coverslips should be counted and classified thus identifying all the cells in a single drop of blood. Since this is impractical a representative sample is examined. An area of good cell distribution (Chapter 1, page 27) is selected under low-power magnification

**Table 6-3. The 95% Confidence Limits for Differential Leukocyte Counts**

Percentage of Given Cell Type	100 Cells Counted	200 Cells Counted
0	0.0- 3.0	0.0- 1.5
1	0.5- 4.7	0.2- 3.1
2	0.4- 6.3	0.7- 4.6
3	0.8- 7.7	1.3- 5.9
4	1.4- 9.1	2.0- 7.2
5	2.0-10.5	2.7- 8.5
10	4.0-16.0	5.8-14.2
20	12.0-28.0	14.3-25.7
30	20.8-39.2	23.5-36.5
40	30.2-49.8	33.1-46.9
50	40.0-60.0	42.9-57.1
60	50.2-69.8	53.1-66.9
70	60.8-79.2	63.6-76.5
80	72.0-88.0	74.3-85.7
90	84.0-96.0	85.8-94.2

For percentages of 5 or less the limits were derived assuming a Poisson rather than a binomial distribution.

From Cartwright,<sup>11</sup> courtesy of the author and Grune & Stratton

(100 $\times$ ) and this area is then examined in detail under oil-immersion magnification (approximately 1000 $\times$ ). The numbers of neutrophils, eosinophils, basophils, lymphocytes, monocytes, and other leukocytes, if present, are recorded and the proportion of each is calculated. The error in such estimates of cell percentage is considerable because only a very small sample (100 or 200 cells) of a large population (more than 5000) is examined.

Distributional errors are reduced as more cells are enumerated. Confidence tables or curves from which one may estimate the probable error of a differential count when various numbers of cells are counted are available.<sup>150,153</sup> Table 6-3 shows 95% confidence limits. It is evident that as more cells of a given type are counted and as the total number of cells enumerated increases, the accuracy of the differential count is greater. Thus, if 200 cells are counted and a frequency of 70% is found for a given cell type, the true value can be expected to lie between 63.5 and 76.5% for 95% of such counts. If a subsequent 200-cell differential count gave

a figure of 80% for that cell type, this would indicate that the difference is probably real, while if only 100 cells had been counted the difference would probably not be significant. Even so, if one is dealing with cells present only in small numbers (such as eosinophils or basophils in the usual smear) the values obtained from the differential count provide only a gross estimate of cell frequency. For more accurate enumeration of these cell types, absolute counting methods have been developed<sup>663,674,720,731</sup> (see pages 263, 266).

From the total leukocyte count (Chapter 1) and the differential count the absolute concentration of each leukocyte type can be calculated. The accuracy of the result depends on the validity of the total leukocyte count and the differential count. With present-day, automatic cell counters (see Chapter 1) the major component of error now lies in the differential count.

Normal values for absolute leukocyte concentrations are shown in Table 6-4. The absolute leukocyte concentration provides a more accurate picture than the differential count because, in essence, each leukocyte type is a separate cell system with its own functions, control mechanisms, and responses to disease processes; eg, a patient with chronic lymphocytic leukemia whose total leukocyte count is  $100 \times 10^9$  cells/l, 7% of which are neutrophils and the remainder lymphocytes, does not have granulocytopenia. His blood neutrophil concentration is  $7.0 \times 10^9$  cells/l, his problem is marked lymphocytosis.

Various "systems" for differential counting were employed at one time<sup>151</sup> (Fig. 6-7). Arnett,<sup>9</sup> for example, painstakingly recorded, and tabulated from left to right, the number of neutrophilic leukocytes with 1, 2, 3, etc lobes and made other subdivisions. The term "*shift to the left*" is derived from this practice and indicates an increase in the proportion of cells with only one or very few lobes, whereas "*shift to the right*" represents an increase in the proportion of multisegmented forms. Schilling used the term "*regenerative shift*" to refer to the increased proportion of juveniles and myelocytes that appear in the blood in response to an acute process, and "*degenerative shift to the left*" to indicate a

failure to mature as a result of depressed marrow function. He pointed out that when the "*shift*" is of the latter type an increased number of immature forms is found in the blood, but the nuclei of these cells are narrow, T-, V-, or U-shaped, deeply stained, and show little structural detail ("*Stabkernige*" or "*staff*" cells).<sup>152</sup>

From a clinical viewpoint it is now appreciated that it is useful to determine whether or not young forms of neutrophils (band forms and younger) are increased and whether there is an increased proportion of multinucleated forms. An increase of younger forms (band cells, metamyelocytes, and myelocytes) ("*shift to the left*") suggests increased release of young neutrophils from the bone marrow; this is seen in association with acute infections<sup>50</sup> and inflammation (see Chapter 41). If there appears to be a "*shift to the right*" it may be useful to make a *neutrophil lobe count*. This involves counting the total number of nuclear lobes in 100 or 200 neutrophils, calculating the average lobe number per neutrophil, and comparing the results with normal values. The chief difficulty here is clear definition of what constitutes a separate lobe (see page 231). If complete separation of nuclear lobes with or without a connecting filament is the definition used, the normal mean neutrophil lobe count is 2.04, with 95% of normal values falling between 1.66 and 2.42.<sup>53</sup> An increase in mean neutrophil lobe count suggests vitamin B<sub>12</sub>

**Table 6-4. Absolute Blood Leukocyte Concentrations in 291 Normal Adults\***

Cell Type	Median	95% Limits
Total leukocytes	7.0	4.3-10.0
Band neutrophils	0.52	0.1-2.1
Segmented neutrophils	3.0	1.1-6.05
Total neutrophils	3.65	1.83-7.25
Lymphocytes	2.5	1.5-4.0
Monocytes	0.43	0.2-0.95
Eosinophils	0.15	0-0.7
Basophils	0.03	0-0.15

\*Values expressed in cells  $\times 10^9$ /l. Leukocyte counts performed with an electronic counter (Coulter model A or F). At least 200 cells were enumerated for each differential count.

From Orfanakis et al,<sup>53</sup> courtesy of the authors and The American Journal of Clinical Pathology

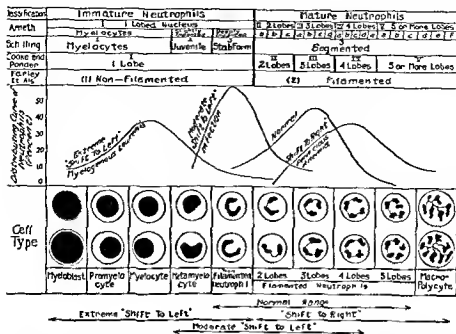


Fig. 6-7. Diagram illustrating several classifications of the neutrophils. Note that all the classifications agree on a common dividing line between mature and immature cells. The Schilling classification further subdivides only the immature cells. The Cooke and Ponder and the Arneith classifications further subdivide only the more mature cells. (From Haden<sup>151</sup>)

or folic acid deficiency, congenital hypersegmentation of neutrophils, or renal disease.<sup>33</sup> A ratio of five-lobed to four-lobed polymorphonuclear cells that is greater than 0.17 was said to be more regularly associated with  $B_{12}$  deficiency than is an increase in mean nuclear lobe count.<sup>28</sup>

Alterations in the total number of leukocytes and in their relative proportions are of considerable significance as measures of the reactions of the body to noxious agents. Thus, in addition to noting the presence of young or multilobed neutrophils, the presence of increased numbers of promonocytes<sup>111</sup> or "stimulated" lymphocytes in the blood smear is indicative of certain disease processes. The reactions of leukocytes in disease will be discussed in Chapters 41 and 42 as will the presence of abnormal inclusions such as "toxic" granulation, Döhle bodies, and various inherited abnormalities in leukocyte morphology.

An additional special type of differential cell count which is in common use is the histochemical, semiquantitative estimate of the leukocyte alkaline phosphatase content of neutrophils.<sup>38</sup> In this procedure a fresh blood

smear is overlaid with an incubation mixture containing a substrate such as naphthol AS-BI phosphate, which is hydrolyzed by the alkaline phosphatase present in the normal "specific" neutrophil granules (see Chapter 1, page 28). The hydrolyzed product is coupled to a salt to form an insoluble red dye (Plate XV) and the intensity of the staining is graded 0 to 4+. After grading 100 neutrophils and recording the numbers of cells graded 0, 1+, 2+, etc, the product of the number of cells in each category and the category number (eg,  $2+ \times 20$  cells) is obtained, and the sum of these products is totaled. A maximum score is 400 and the usual normal score is about 25 to 140.<sup>18</sup>

### Leukocyte Kinetics (Dynamics of Production, Circulation, and Turnover) and Cell Function

Although the role of leukocytes in the defense of the organism has been known since the studies of Metchnikoff in 1892, and it is evident that each of the several leukocyte cell

lines has special properties and a unique role in body defense, the details and mechanisms by which the functions of leukocytes are accomplished still are incompletely understood. Basic to the accomplishment of the roles are cell multiplication, maturation and storage, and delivery via the blood to the tissues and sites of infection or cell damage. These processes are referred to as *leukocyte kinetics* and are different for each leukocyte type. It will simplify the discussion to consider each type of leukocyte as a separate system, but it must be realized that the several systems constantly interact and complement one another in the defense of the body.

### Neutrophil Series—Kinetics, Properties, and Functions

In Chapter 2 the process of mitotic cell division, the cell generation cycle, and the origin of neutrophils and other cell types from a multipotential colony-forming unit (CFU) cell were discussed. The CFU cell is thought to produce a "committed" stem cell (CSC) from which in turn the myeloblast is formed and from this the neutrophilic series is derived. The production, kinetics, and

life span of the neutrophil have been the subject of a number of reviews.<sup>176,181,186,189,190,191,213,252,258,279,260,263,282,284</sup> A model describing these processes is shown in Figure 6-8.

### The Mitotic and Maturation Compartments—Kinetics of Production and Turnover

Neutrophil production in normal adult man appears to take place only in the bone marrow. The life cycle of the neutrophil can be divided conveniently into bone marrow, blood and tissue phases. It is assumed that cells move through the system in a more or less orderly manner as if in a pipeline; this view is supported by the progressive movement of isotopic tracers<sup>70,195,247,259</sup> and azurophilic granules<sup>10,13,73</sup> through the system.

The myeloblast, promyelocyte, and myelocyte are capable of cell division, as judged by direct observation in cultures<sup>14</sup> and by their ability to incorporate tritiated thymidine into their nuclear DNA.<sup>182</sup> These forms therefore constitute the *mitotic compartment* (Fig. 6-8). They simultaneously undergo differentiation, as evidenced by the appear-

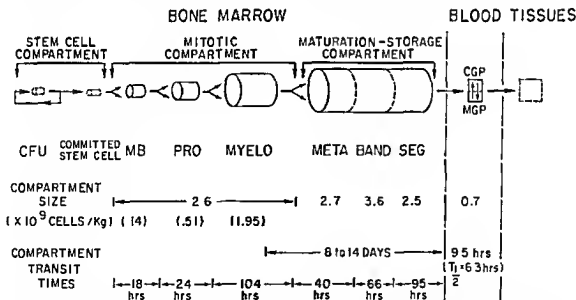


Fig. 6-8 Model of the production and kinetics of neutrophils in man. The marrow<sup>203</sup> and blood<sup>184</sup> compartments have been drawn to show their relative sizes. The compartment transit times as derived from DF<sup>33</sup>P studies<sup>184,282</sup> are shown on the next to the last line. Values derived from tritiated thymidine studies are shown on the last line.<sup>191</sup>

ance of azurophilic and specific granules in their cytoplasm. The more mature forms of the neutrophil series (metamyelocyte, band, and polymorphonuclear neutrophil) are usually considered to be incapable of cell division (except perhaps in unusual circumstances<sup>245</sup>) and do not incorporate tritiated thymidine into their nuclei, but they do exhibit continuing maturational changes and thus constitute the *maturation compartment*. From the maturation compartment, cells flow into the blood where they are distributed in two sites: (1) the circulating blood granulocyte pool (CGP), or (2) the marginal granulocyte pool (MGP), where they adhere to the walls of postcapillary venules. Cells in these two sites are in continual equilibrium. Eventually the cells move through vessel walls to enter the tissues.

In such a scheme, cell production can be estimated either by assessing the production ("birth") rate in the mitotic compartment or by measuring cell flow through subsequent stages such as the blood. These measurements are facilitated if the system is studied in the steady state when compartment sizes are constant and cell flow reflects net production and destruction.<sup>243</sup>

It is apparent that if one assumes a steady state in a scheme such as that shown in Figure 6-8, the flow of cells out of any pool ( $K_{out}$ ) is equal to the flow of cells into that pool ( $K_{in}$ ) plus any cells produced ("born") in the pool ( $K_b$ ); thus

$$K_{out} = K_{in} + K_b$$

Obviously, in pools other than those in the mitotic compartment,  $K_b$  is zero, and measurements of cell flow ( $K_{in}$  or  $K_{out}$ ) will equal effective cell production provided no cell death occurs within the pool.

### Production in the Mitotic Compartment

Since myeloblasts, promyelocytes, and myelocytes comprise about 0.9%, 3.3%, and 12.7% of the marrow cells, respectively (Table 2-1, page 62), it has been assumed that there are four or five divisions in the system.<sup>232</sup> From blood neutrophil radioac-

tivity curves obtained after DF<sup>32</sup>P injection into man it was suggested that there must be at least three divisions at the myelocyte stage.<sup>232</sup> It was also suggested from marrow differential counts that only four or five divisions occur in the entire neutrophil proliferation scheme.<sup>232</sup> This is in agreement with experiments in dogs<sup>261</sup> and with data from model studies.<sup>238</sup> In contrast, studies of myeloid islands in the rat thymus provided evidence for seven divisions during granulocytogenesis: one in the myeloblast stage, two in the promyelocyte stage, three in the myelocyte stage, and a final one in the metamyelocyte stage.<sup>274,275</sup>

Calculations made from *mitotic index* data<sup>191,210,232,233,234,240</sup> provide estimates of cell generation time ( $t_g$ ) and pool turnover time. *Mitotic index*<sup>210,240,241</sup> is defined as:

$$MI = \frac{N_m}{N}$$

where MI is the mitotic index for any morphologic cell pool,  $N_m$  is the number of mitoses in that pool, and  $N$  is the total number of cells in the pool. MI can also be expressed as the ratio of the time spent in mitosis ( $t_m$ ) to the cell generation time ( $t_g$ ):

$$MI = \frac{t_m}{t_g}$$

Combining both definitions:

$$MI = \frac{N_m}{N} = \frac{t_m}{t_g}$$

It is apparent that, by providing determined values for mitotic index and mitotic time in the last equation, the generation time ( $t_g$ ) for a particular cell pool can be approximated. From  $t_g$  and the pool size ( $N$ ) the birth rate,  $K_b$ , can be obtained if all cells in the pool are in cycle, since each mitosis gives rise to one new cell:

$$K_b = \frac{N}{t_g}$$

In effect the cell birth rate is equal to the number of mitoses occurring per unit time, or

$$K_b = \frac{N_m}{t}$$



Although the concept is simple, several problems arise.<sup>232,233</sup> A major one is that the morphologic boundaries of most cell pools are not clearly delineated in terms of the cell cycle.<sup>233</sup> For example, to calculate cell production in the myelocyte pool it must be assumed that all myelocytes are destined to divide; that is, that there are no cells recognized as myelocytes that are not going to divide again. Since the daughter cells of the last myelocyte mitosis almost certainly do not suddenly become metamyelocytes on completion of division,  $N_m$  in the above equation will be erroneously large and estimates of  $t_c$  will be erroneously long.

If the fraction of nonmitotic cells in the myelocyte population were known, the calculations could be corrected for this error, and this has been attempted.<sup>186</sup> A second major problem is the fact that values for the mitotic index have varied from 6.93 to 43 per 1000 nucleated cells in man,<sup>193,200,210,225,232,240</sup> 28% to 48%<sup>210,228,232,250</sup> of these being in the leukocytic system. In addition, there appears to be considerable diurnal variation in the mitotic index in man<sup>232,250</sup> as well as in animals.<sup>219</sup> Finally, in order to calculate the absolute neutrophil production rate (in cells per unit of time) the size of the marrow cell pool under consideration must be known. Methods for measuring the sizes of marrow myeloid pools have been developed<sup>203,204</sup> (see page 248), but to date no one has measured these sizes and mitotic index in the same animal at the same time and then calculated neutrophil production rate. Nevertheless, values for the mitotic index for each of the mitotable neutrophil precursors have been determined,<sup>232,240</sup> and within the assumptions inherent in such calculations<sup>233</sup> neutrophil production has been estimated.<sup>234,258</sup>

Similar calculations of neutrophil production can be made from *tritiated-thymidine-labeling index* data. After flash labeling with <sup>3</sup>HTdR, autoradiographs of the bone marrow have been made and the proportion of nucleated cells that have incorporated the label into their nuclei has been determined.<sup>189,260</sup> This "labeling index" (LI) represents the ratio of labeled cells,  $*N$ , or cells in DNA

synthesis ( $N_s$ ) to total cells ( $N$ ) of a defined morphologic type:

$$LI = \frac{*N}{N} = \frac{N_s}{N}$$

The labeling index can also be defined in terms of DNA synthesis time ( $t_s$ ) and the cell generation time ( $t_c$ ) since <sup>3</sup>HTdR is taken into the cell only during the period of DNA synthesis; thus:

$$LI = \frac{t_s}{t_c}$$

By combining both definitions we have:

$$LI = \frac{N_s}{N} = \frac{t_s}{t_c}$$

and from determined values for LI and  $t_s$ , the generation time and turnover of a given cell population can be estimated. As with mitotic index data, birth rate is a function of the population turnover time which can be approximated from the generation time or time spent in various phases of the cell cycle:

$$K_b = \frac{N}{t_c} = \frac{N_s}{t_s}$$

Some of the same problems arise with the tritiated-thymidine-labeling index that are encountered in the use of the mitotic index.<sup>194,233</sup> In addition, the use of labeled compounds raises questions of label reutilization<sup>207,208</sup> or elution<sup>194</sup> and perturbation of the cell population by the compound<sup>170,216,220,222,261</sup> or by its radioactivity,<sup>225,272</sup> to cite just a few.<sup>194</sup>

The labeling indices reported for man are: myeloblast—0.85; promyelocyte—0.65, and myelocyte—0.33.<sup>189</sup> Somewhat different values have been reported in dogs<sup>260</sup> and rats.<sup>240</sup> By using the labeling indices for man and a value for  $t_s$  of five hours (based on studies in dogs) and by determining relative compartment sizes for each cell type from the bone marrow differential count, the relative birth rates ( $K_b$ ) of cells have been calculated.<sup>189,240</sup>

While some have found good agreement between neutrophil production as calculated from mitotic index and from labeling in-

dex,<sup>240</sup> considerable discrepancy has been reported by others.<sup>186</sup> This may be because: (1) the mitotic index values obtained were low, (2) the studies were done in different subjects at different times, and/or (3) too small a value for  $t_s$  was used in the calculations.

The turnover time of a labeled compartment and neutrophil production rate also may be estimated by measuring the *grain count halving time*.<sup>195</sup> This method will give the generation time only if each cell in a given class divides and if there is no label feeding into the compartment from a labeled precursor class or as a result of label reutilization.<sup>187</sup> If any of these criteria are not met the half-time for grain count decrease will be longer than the true value and the estimate of generation time is then only a maximal value. Additional disadvantages of this method are that at least several bone marrow samples distributed throughout several half-times are needed, and grain counting is extraordinarily tedious and subject to considerable error. Nevertheless, estimates of compartment

turnover time have been made with this method using  $^3\text{HTdR}$ <sup>195</sup> and radiolabeled sulfate.<sup>70</sup>

After flash labeling with  $^3\text{HTdR}$  the cohort of cells labeled during DNA synthesis may be followed as it subsequently enters mitosis and the time course of labeled mitoses can be recorded.<sup>187,279</sup> Theoretically such curves should permit measurement of the post DNA synthesis gap ( $G_2$ ), mitotic time ( $t_m$ ), DNA synthesis time ( $t_s$ ), cell generation time ( $t_c$ ), and pre DNA synthesis gap ( $G_1$ ) (see page 42, [Fig. 2-1] and Fig. 6-9). In actual practice, biologic variation rounds off the *percent labeled mitosis curves*, and rapid damping of the waves of cells passing through mitosis (Fig. 6-9) renders such measurements less precise than would be ideal. However, estimates of myeloid DNA synthesis time obtained with this method are about 11 to 13 hours in man<sup>187,279</sup> and are in good agreement with estimates made in gastrointestinal mucosal cells. From the level of the damped plateau reached after a few hours, the ratio  $t_s/t_c$  can be obtained (Fig. 6-9) and from this the generation time can be calculated. If one

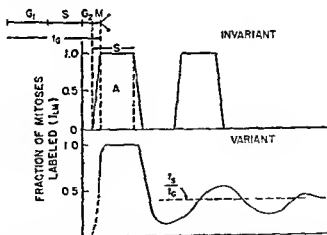


Fig 6-9. Schematic diagram of the percentage of labeled mitoses in the course of cell generation. The top portion of the diagram shows the theoretical configuration that would be seen if cells flowed through the proliferation cycle with no variation. The effects of moderate variation in time spent in the several cycle phases on the percent labeled mitosis curve are shown in the bottom portion.  $G_1$  is pre-DNA synthesis resting phase (gap),  $S$  is the DNA synthetic period,  $G_2$  is the post DNA synthesis gap and  $M$  is mitosis.  $t_s$  is the time spent in  $S$ , and  $t_c$  is the duration of the entire generative cycle. (From Cronkite,<sup>187</sup> courtesy of the author, the Brookhaven National Laboratory and the National Cancer Institute.)

assumes that the generation time and compartment transit time are the same or if one knows the proportion of cells in a compartment that is actively proliferating, the neutrophil production rate can be calculated.

#### Neutrophil Production as Measured by Cell Flow in Other Compartments

Still another method for approximating neutrophil production is to follow the *appearance of  $^3\text{HTdR}$ -labeled cells in the metamyelocyte compartment*. Since metamyelocytes do not divide or take up  $^3\text{HTdR}$ , the appearance of labeled cells in this compartment should reflect the flow of cells into it from the myelocyte compartment; in the steady state this should also reflect the turnover of the metamyelocyte compartment and thus cell production. There is about a three-hour lag after the injection of  $^3\text{HTdR}$  before label appears in metamyelocytes both in dogs<sup>260</sup> and in man<sup>137</sup>; this time interval represents the minimum time for myelocytes taking up the label to pass through  $G_2$  and mitosis and to become metamyelocytes (see Chapter 2, page 42). After this lag, the rate of labeled cell inflow into the metamyelocyte compartment is about 3 to 5% per hour in both species.

In the dog, cell inflow into the metamyelocyte compartment measured in this fashion was less than 50% of that calculated from labeling index data.<sup>260</sup> This led Patt to suggest that there is a major component of ineffective granulocytopoiesis, a "*myelocyte sink*," in the normal animal. However, similar calculations in man have not confirmed the studies in dogs.<sup>186</sup> The resolution of this enigma will require the simultaneous measurement of cell production using several methods in the same animal at the same time.

Neutrophil production also can be approximated by measuring the flow of cells through the blood, the *blood granulocyte turnover rate* (GTR). Diisopropylfluorophosphate ( $\text{DF}^{32}\text{P}$ ) binds irreversibly with a number of esterase enzymes and has been shown to label neutrophils primarily.<sup>183,231</sup> By means of this label the total blood granulocyte pool (TBGP) can be measured (see below) and the rate of dis-

appearance of labeled neutrophils from the blood can be determined.<sup>232</sup> Since neutrophils leave the blood in a random manner (exponential disappearance curve) the GTR is calculated as follows from the total blood granulocyte pool (TBGP) and the  $t_{1/2}$ :

$$\text{GTR} = \frac{.693}{t_{1/2}} \times \text{TBGP}$$

where .693 is the natural logarithm of 2, and  $t_{1/2}$  is the blood neutrophil half-disappearance time. If there is no significant component of neutrophil death in the marrow, the blood GTR will equal total neutrophil production. If there is significant neutrophil death in the bone marrow, the blood GTR measures *effective neutrophil production* and the difference between this and total neutrophil production is *ineffective granulocytopoiesis*. Measurements of neutrophil production by this method gave values ranging from 62 to  $400 \times 10^7$  neutrophils/kg/day in man<sup>174</sup> (Table 6-5) and 150 to  $560 \times 10^7$  neutrophils/kg/day in dogs.<sup>260</sup>

Of the above methods for assessing neutrophil production only the measurement of blood neutrophil turnover rate with  $\text{DF}^{32}\text{P}$  can be carried out with sufficient facility to be of use in studying groups of patients in a clinical setting, and even this is possible in only a few research centers.

#### Size of the Marrow Compartments and Their Morphologic Subdivisions

In all of the above procedures except the  $\text{DF}^{32}\text{P}$  measurements it is necessary to know the number of marrow myeloid cells under study in order to calculate neutrophil production. In the absence of such data only calculations of relative cell production are possible.<sup>186</sup> Direct measurements of the volume and cellularity of the marrow have been made in rats,<sup>230</sup> mice,<sup>257</sup> and guinea pigs,<sup>223,224</sup> but such studies are not possible in man. The  $^{59}\text{Fe}$  dilution technique for measuring the marrow nucleated red cell mass, from which myeloid mass can be calculated by using the myeloid:erythroid ratio, provides the best estimates of marrow cellularity for man and several other spe-

cies.<sup>203,204,257,280</sup> The mean values obtained in nine male patients undergoing thoracotomy for tuberculosis are presented in Figure 6-8. Values determined in rats, rabbits, and monkeys also have been reported.<sup>201</sup>

### Transit Time through the Nondividing Maturation Pool

Following a pulse label of tritiated thymidine or radiophosphate there is a delay of several days before labeled segmented neutrophils appear in the blood. This represents the minimum time from DNA synthesis in the last myelocyte generative cycle until the cell has matured into a segmented neutrophil (or band form) and is released into the blood. In patients in a "normal" steady state this minimum transit time or *emergence* time was between 96 and 144 hours<sup>213,264</sup>; in patients with infection it was as short as 48 hours. Emergence time in dogs is 2 to 3 days<sup>215</sup> and in the rat 36 to 42 hours.<sup>211</sup>

The mean value for *myelocyte-to-blood transit time* after DNA labeling (the time from <sup>3</sup>HTdR or radiophosphate injection to the peak of the blood granulocyte radioactivity curve) is 6 to 9 days in hematologically "normal" convalescent patients. In contrast, studies in which DF<sup>32</sup>P was injected intravenously into normal volunteers (prisoners) led to the conclusion that the mean myelocyte-to-blood transit time was 11 days with a range of 8 to 14 days.<sup>184,282</sup> Studies using <sup>3</sup>HTdR and DF<sup>32</sup>P simultaneously in the same subjects demonstrated that these discrepancies reflected differences between normal, ambulatory subjects and hematologically "normal," convalescent patients.<sup>172</sup> In dogs the average myelocyte-to-blood transit time was 5 days as measured with both <sup>3</sup>HTdR and DF<sup>32</sup>P.<sup>172</sup>

### Manner of Segmented Neutrophil Release from the Marrow into the Blood<sup>277</sup>

After <sup>3</sup>HTdR injection and at the time when label was first seen in band and segmented neutrophils in the bone marrow, some labeled cells were also seen in the

blood.<sup>189</sup> From this it was suggested that the release of band or segmented neutrophils from the marrow does not follow a strict pipeline or first-in-first-out sequence.<sup>189,213</sup> However, it is not clear whether these observations reflected variance around a mean transit time,<sup>178,248,282</sup> or random release of mature neutrophils from the marrow.<sup>213</sup> Studies in dogs strongly favor the mean transit time concept rather than random release.<sup>218</sup>

### Neutrophil Kinetics in the Blood

For a long time it has been accepted that neutrophils emigrate from the blood and are continuously replaced,<sup>271</sup> but it has not usually been appreciated that a large proportion of granulocytes in the blood are not in the main stream of the circulation. It is well known that various physiologic influences such as exercise, excitement, and other factors may be associated with a substantial increase in the leukocyte count,<sup>309</sup> and this was long attributed to release of leukocytes sequestered in the spleen. However, various observers have recorded the fact that such changes also occur in splenectomized persons.

### DF<sup>32</sup>P Studies

With the development of the DF<sup>32</sup>P-labeling technique, in which neutrophils are labeled *in vitro* with DF<sup>32</sup>P and the undamaged cells are returned to their donor, it was demonstrated that there are two "pools" of neutrophils in the blood, a circulating granulocyte pool (CGP) and a marginated granulocyte pool (MGP).<sup>165,168,252</sup> In normal man, neutrophils in these two sites are in constant equilibrium and the pools are of about equal size (Table 6-5). The CGP is calculated from the blood leukocyte count and the blood volume. TBGP is measured by the dilution of DF<sup>32</sup>P-labeled neutrophils after their re-injection.<sup>165</sup> MGP is the difference between TBGP and CGP.

Brief exercise or epinephrine injection increases the size of the circulating granulocyte pool by about 50%, but the TBGP is unchanged; the neutrophilia so produced reflects

Table 6-5. Blood Neutrophil Kinetic Parameters in 71 Normal Subjects

	Mean	95% Limits
TBGP (cells $\times 10^7$ /kg)	61	27-128
CGP (cells $\times 10^7$ /kg)	31	13-49
MGP (cells $\times 10^7$ /kg)	29	8-115
$T_{1/2}$ (hours)	6.3	4-10
GTR (cells $\times 10^7$ /kg/day)	160	62-400

Based on data from Bishop et al.<sup>174</sup>

a demargination of cells which lasts only a few minutes.<sup>165</sup> The location of the margined cells is thought to be along the walls of small blood vessels, primarily postcapillary venules, in many body tissues.<sup>281</sup> The distribution of cells in the CGP and MGP can be altered by other means. For example, after endotoxin injection a transient neutropenia was noted at 90 minutes but the TBGP was not significantly changed; thus there was mainly a shift from CGP to MGP. At the end of five hours, there was a marked increase in the TBGP as a result of an outpouring of cells from the bone marrow, and the CGP and MGP were of about equal size.<sup>166</sup> The administration of steroids also produced an increase in the size of the TBGP, in part for the same reason but also because of decreased outflow into the tissues.<sup>173</sup>

After the return of DF<sup>32</sup>P-labeled neutrophils to their donor the disappearance of labeled cells from the blood follows a single exponential curve with a *half-disappearance time* ( $t_{1/2}$ ) of about seven hours in most normal subjects.<sup>161,167,168,184,217,252</sup> This implies that neutrophils are destroyed or leave the blood randomly rather than according to their age (senescence), as is the case for erythrocytes and platelets.<sup>252</sup> Confirmation of this is found in the fact that <sup>3</sup>HtDR-labeled neutrophils appear in the blood and in saliva almost simultaneously.<sup>212</sup>

From leukopheresis<sup>295</sup> and other experiments<sup>251</sup> it is probable that neutrophils that have crossed the endothelial barrier between blood and tissues do not reenter the circulation, at least in significant numbers. The number of neutrophils that pass through the blood each day has been found to be

$160 \times 10^7$  cells/kg/day (Table 6-5); this is the granulocyte turnover rate (GTR). It has been proposed that neutrophils become senescent and develop pyknotic nuclear lobes as time elapses, this process truncating the exponential curve of disappearance of DF<sup>32</sup>P-labeled neutrophils.<sup>212</sup> Although this seems plausible, no truncation of DF<sup>32</sup>P curves has been noted. Perhaps this is because pyknotic, senescent cells constitute only 0.5% of neutrophils and detection of their early removal could be easily missed.

### <sup>51</sup>Cr Studies

Leukocytes can also be labeled in vitro with radiochromate (<sup>51</sup>CrO<sub>4</sub>) and then returned to the body.<sup>205,254,255</sup> Unlike DF<sup>32</sup>P, which labels mainly granulocytes, leukocyte types other than neutrophils are labeled by this technique.<sup>201,205,206,255,256,264</sup> Under some circumstances (eg, in chronic lymphocytic or chronic myelocytic leukemia) this is advantageous since leukocytes other than neutrophils can be labeled and their distribution and turnover followed.<sup>201,205,256</sup> In addition, <sup>51</sup>Cr is a gamma-emitting isotope and thus external counting techniques should be helpful in locating sites of sequestration or destruction. After the infusion of autologous, radiochromate-labeled leukocytes into hematologically normal subjects the proportion of cells recovered in the blood is considerably less than that of DF<sup>32</sup>P-labeled neutrophils.<sup>255</sup> This may reflect early elution of part of the label,<sup>270</sup> or perhaps the rapid disappearance of <sup>51</sup>Cr-labeled mononuclear cells. However, after an initial rapid decrease, curves of <sup>51</sup>Cr-labeled leukocyte radioactivity in the blood have given  $t_{1/2}$  values quite comparable to those obtained with DF<sup>32</sup>P. No significant organ sequestration of labeled leukocytes has been detected in normal subjects.<sup>255</sup>

### Neutrophil Migration into Tissues and Sites of Destruction

The sites into which neutrophils normally disappear are poorly understood. Labeled blood neutrophils are found in oral saliva,<sup>211</sup>

but loss into saliva may reflect subclinical infection since few if any cells are found in the salivary ducts<sup>227</sup>; the rate at which granulocytes enter the oral cavity has been correlated with the degree of gingivitis.<sup>236</sup> Nevertheless, some cells do penetrate the oral mucosa in healthy persons, presumably as a result of diapedesis.<sup>227</sup> Loss of leukocytes in the urine also has been demonstrated in normal subjects<sup>160</sup>; this increases markedly in pyelonephritis.<sup>218</sup> In addition, arteriovenous catheterization studies in dogs<sup>162</sup> and man<sup>171</sup> have provided evidence that suggests that leukocytes also are removed through the lungs, in the liver and spleen. Significant numbers may be lost into the gastrointestinal tract. No quantitative data concerning the rate of loss through these various organs are available. That loss via subclinical infections at the various body surfaces is not a major factor in neutrophil turnover is suggested by the observation that bone marrow and blood leukocyte counts were essentially the same in germ-free and in normal mice.<sup>179,214</sup>

At a local site of tissue damage or infection, adherence of neutrophils to the endothelial cells of the vessel wall and their subsequent emigration into the tissues can be seen within minutes. Although it has been suggested that chemotaxis offers the best explanation for the *adherence and emigration*, there is no proof that this is the case.<sup>458</sup> After the initial sticking the neutrophils can be seen to project pseudopods between the endothelial cells and to force a passage between them.<sup>434,446</sup> Further migration is then delayed by the basement membrane and periendothelial cells, and the neutrophils may move parallel to but beneath the endothelium until a passage into the surrounding connective tissue is found. There is no evidence of neutrophil granule lysis to suggest that a passage is made by dissolving interendothelial cell cement substance or collagen fibrils and there is no consistent morphologic evidence of change in the endothelial cells.<sup>434</sup> Whether similar mechanisms operate under normal conditions is not known. Once neutrophils leave the blood they do not return in significant numbers.<sup>177,231</sup>

### Normal Values and Physiologic Variations

The changes in blood leukocyte concentration that occur with growth and development were described in Chapter 2 and shown in Figure 2-11. By the age of four to eight years the blood differential cell count approaches that seen in the adult. Normal values for adults are presented on page 242. These values were determined by using anticoagulated blood and electronic counters, but the results are essentially identical to those obtained by hemocytometer methods over 30 years ago.<sup>321</sup> Metamyelocytes or myelocytes are not often seen on routine examination of the blood smear, but a few such cells can be found in normal blood by careful search or, more readily, by examination of buffy coat smears (3.6/3000 granulocytes)<sup>299</sup>; atypical mononuclear forms and megakaryocyte fragments containing nuclei are seen in such smears also. Whether there is a significant difference in leukocyte concentration between the sexes or with *advancing years* is not clear since reports are conflicting.<sup>290,303,319,329</sup> There probably is no difference between *races*<sup>55</sup> but this topic has not been studied extensively.

Although leukocyte concentration is maintained within definite limits in normal man, fluctuations occur during a single day as well as from day to day and periodic cycling at longer intervals has been reported<sup>315</sup> (see below). The suggestion that there is a characteristic hourly rhythm has not been confirmed<sup>294,310</sup> nor has the occurrence of a "digestive" leukocytosis been established.<sup>333</sup> Light influences the diurnal variation.<sup>327</sup> Under conditions of complete *physical and mental relaxation* a basal level of 5.0 to  $7.0 \times 10^9$  cells/l is usual.<sup>306</sup> Ordinary *activity* may be associated with a moderate increase and a somewhat higher level is common in the afternoon. Under all these conditions, however, the leukocyte count tends to remain within the range of "normal" (Table 6-4).

Conclusive demonstration of the effects of *climate or season* on the leukocyte count is lacking. It was claimed that meteorologically

conditioned fluctuations occur.<sup>324</sup> Heat and intense solar radiation are said to cause leukocytosis.<sup>309</sup> Artificially induced heat causes lymphocytosis.<sup>302</sup> Sunlight and ultraviolet radiation are reported to cause lymphocytosis. Acute *anoxia*, both anoxic and anemic, causes leukocytosis.<sup>297</sup> This is neutrophilic in type and does not develop in adrenalectomized rats. In the first few days after an individual has arrived at a high altitude, some leukocytosis, accompanied by lymphopenia and eosinopenia, has been observed. Slight lymphocytosis and eosinophilia soon develop, however.<sup>332</sup>

Marked leukocytosis regularly occurs with *strenuous exercise*. Counts as high as  $22.0 \times 10^9/l$  have been recorded for a runner after making a 100-yard dash in 11 seconds, and  $35.0 \times 10^9/l$  on completing a quarter-mile run in less than a minute.<sup>306</sup> The increment of cells usually is made up mainly of segmented neutrophils, but lymphocytosis may be prominent as well. Such leukocytosis recedes to normal in less than an hour and in the case of the neutrophils is due to a shift of cells from marginal sites (MGP) to the circulation (CGP) ("*shift*" leukocytosis).<sup>165,166</sup> This leukocytosis occurs in the absence of the spleen and thus the spleen is not a major site of cell margination. Leukocyte counts above  $20.0 \times 10^9/l$ , mainly neutrophils, are regularly recorded for runners completing a marathon of 26 miles in two and one-half to three hours; there is disagreement as to whether a shift to the left, suggesting mobilization of marrow neutrophils, occurs in this circumstance.<sup>306</sup> Postmarathon run leukocytosis subsides slowly over a number of hours and probably reflects a redistribution of granulocytes in the blood, as mentioned above, combined with mobilization of cells from the marrow with an increase in TBGP size. The magnitude of the leukocytosis associated with exercise appears to depend primarily on the intensity of the activity rather than upon its duration.<sup>300</sup>

*Convulsive seizures*, from whatever cause, are associated with increases in leukocyte count similar to those following violent exercise. Electrically induced convulsions are fol-

lowed by a reduction in eosinophils and lymphocytes and a rise in neutrophils, findings interpreted as consistent with the effects of adrenal hormone secretion.<sup>291</sup> *Epinephrine* injection produces a leukocytosis the nature and duration of which appear to vary with the mode of administration. Intramuscular injection causes leukocytosis in two phases<sup>304,326</sup>; in the first, which is maximal at 17 minutes, the number of neutrophils, lymphocytes, and eosinophils increases and subsequently returns toward normal over several hours. This almost certainly represents a shift leukocytosis. In the second phase the number of neutrophils rises again at about four hours, while the number of lymphocytes and eosinophils remains at or below pre-injection levels<sup>326</sup>; this phase may reflect an adrenal steroid effect and consists of an absolute neutrophilia. After intravenous injection a brief leukocytosis peaking at 5 to 10 minutes and of total duration of less than 20 minutes occurs and has been shown to be purely a shift neutrophilia.<sup>165,166</sup> The leukocytosis which follows subcutaneous injection is more variable.

During attacks of *paroxysmal tachycardia*, leukocytosis with cell counts of  $13.0$  to  $22.0 \times 10^9$  cells/l has been reported.<sup>312</sup> *Pain*, *nausea* and *vomiting*, and *anxiety* may cause leukocytosis in the absence of infection.<sup>314</sup> The lack of an increase in the number of band forms and metamyelocytes indicates that the neutrophilia is due to the redistribution of the cells between the marginal and circulating pools.

Either *anesthesia* produces leukocytosis; emotional and reflex reactions and struggling during the stage of excitement probably are responsible. Narcosis with barbitol compounds usually reduces the leukocyte count.

During the ovulatory period, eosinopenia and a slight rise in the number of leukocytes as well as increased levels of 17-hydroxycorticosteroids have been reported.<sup>298,322</sup> Slight leukocytosis occurs during *pregnancy*, and neutrophilia increases as term approaches.<sup>295</sup> The onset of labor is accompanied by neutrophilic leukocytosis, which sometimes is very pronounced ( $34.0 \times$

$10^9/l$ ). This continues for a day after delivery, only receding to normal after four or five days. These changes are accompanied by a reduction in the number of circulating eosinophils.<sup>298</sup>

Many of the physiologic variations in leukocytes which have been described above can be explained as manifestations of stimulation of the adrenal cortex. The administration of cortisone or hydrocortisone results in increased blood levels of 17-hydroxycorticosteroids that attain their maximal increase in one hour<sup>318</sup> and are associated with neutrophilia.<sup>307</sup> This is succeeded by eosinopenia and lymphopenia that become maximal in four to eight hours and bear a close relation to the quantity of hormone administered. The neutrophilia was observed to be less constant than the changes in eosinophils and lymphocytes, and it was not well correlated in time or degree to the changes in these cells. These changes probably can be attributed to the decreased efflux of neutrophils from the blood and the increased cell release from the bone marrow,<sup>166,173</sup> mentioned above (page 250) which are associated with the administration of steroid hormones, as well as to their effects on eosinophils and lymphocytes, as described below.

In addition to the above-mentioned short-term changes in neutrophil concentration a long-term oscillation in granulocyte count with a cycle length of 14 to 23 days was reported in 7 out of 11 male subjects.<sup>315</sup> The oscillation amounted to  $\pm 0.50$  to  $0.75 \times 10^9$  cells/l around mean values of between  $2.0$  and  $4.0 \times 10^9$  neutrophils/l. These findings have not been confirmed in studies in our laboratory and elsewhere.<sup>297a,313</sup>

### Control Mechanisms Regulating Granulocytopoiesis

From the above considerations it is evident that a truly steady state exists only for brief periods of time. Shifts of cells between marginal and circulating sites may occur without changes in blood neutrophil turnover,<sup>166</sup> but any change in TBGP size must result from changes in cell inflow and/or egress. Studies

based on the technique of leukopheresis have shown that the normal animal replenishes the TBGP after its depletion by mobilizing cells from the marrow granulocyte reserves.<sup>295</sup> This increase in neutrophil concentration and TBGP size, as well as increases that develop with most bacterial infections or after endotoxin or steroid administration, must be triggered by some signal, and there must also be some means of stimulating cell production to replenish the depleted marrow reserves. Neural mechanisms controlling hematopoietic cell proliferation and release have been suggested<sup>215</sup> as well as neutrophil-regulating substances that may be humoral in nature.<sup>341,364,366</sup> A granulocytosis-promoting factor (GPF) was isolated from a mouse mammary tumor that produced a massive neutrophilia and extramedullary foci of myelopoiesis in the host, but this finding has not yet been confirmed.<sup>348</sup> Endotoxin effects were excluded by suitable controls, and similar activity was found in non-tumor tissue, especially kidney, of several species. It was postulated that GPF may be a naturally occurring granulocytopoietic substance.<sup>348</sup> Partial characterization revealed it to be a micro-molecule ( $<2,000$  mol. wt.) free of protein. It was not found to deplete marrow neutrophil stores and therefore would appear to be different from neutrophilia-inducing factor and leukocytosis-inducing factor mentioned below.

In studies of perfused rat hind limbs the release of neutrophils from the marrow into the blood<sup>353</sup> was increased by a high perfusion flow rate or when the leukocyte content of the perfusate was low.<sup>350</sup> Serum from rats made neutropenic by endotoxin injection was found to be more effective than normal serum in causing neutrophil release, and such a leukocytosis-inducing factor (LIF) was found in the plasma of rats three to four hours following typhoid vaccine administration<sup>351</sup> but not before three hours.

A similar and perhaps identical activity was found in the plasma of dogs recovering from vinblastine- or nitrogen mustard-induced neutropenia or after endotoxin administration in animals<sup>341,342</sup> and in man.<sup>180</sup> The activity



was present during the period of neutropenia and rising neutrophil concentration, but not before or after this period. The activity ("neutrophilia-inducing activity", NIA) was qualitatively dissimilar from that following endotoxin, epinephrine, or cortisone administration and acted by causing release of marrow cells. Dog NIA was found to produce neutrophilia in endotoxin-tolerant, recipient dogs. These studies suggest that there is an endogenously produced humoral factor (NIA or LIF) that causes neutrophil release from the marrow.<sup>342</sup> How this release may be effected is not understood.

Injection of endotoxin has been shown to produce neutrophilia, usually preceded by neutropenia,<sup>340</sup> as discussed earlier (page 250). Detailed studies of the bone marrow during endotoxin-stimulated neutrophil release demonstrated changes in the adventitial cells of the sinusoid wall and marrow interstitium, a decrease in basement membrane substance, and lysosomal disruption in endothelial cells with occasional cell lysis.<sup>369</sup> However, cause and effect could not be distinguished.

Differences in cell deformability that occur with increasing maturity cannot be excluded as influencing the marrow cell release.<sup>46,47,354</sup>

Other studies have demonstrated an inhibitor of marrow neutrophil proliferation (*granulocytic chalone*) in the mature neutrophils and serum of the rat. This could provide a means for negative feedback control of granulocyte proliferation in the mitotic compartment. This activity was found to be specific for the granulocyte system but not species specific. Although of small molecular weight (about 4000) this inhibitor did not act on thymidine metabolism<sup>366</sup> as did a previously reported inhibitor, thymidine phosphorylase.<sup>356</sup> Stimulating activity (antichalone) was also described (mol wt 30,000 to 35,000). In situations of increased demand for neutrophils, chalone in the serum was reduced while antichalone was increased. Both substances appeared to act in the G<sub>1</sub> phase by inhibiting (chalone) or stimulating (antichalone) the entry of neutrophil precursors into DNA synthesis, and both effects were transient and reversible.<sup>367</sup>

Stimulation of granulocyte colony formation in agar plate cultures has been studied extensively.<sup>345,357,360</sup> Such *colony-stimulating activity* (CSA) can be produced by kidney or embryo cell feeder layers, salivary gland, pregnant uterus, and a variety of other tissues such as spleen, liver, lung, brain, testis, and thymus.<sup>346</sup> CSA was present in higher concentration in leukemic AKR mice than in preleukemic mice of the same strain or in other non-leukemic mice.<sup>362</sup> It was present in the sera of mice infected with viruses as well. Germ-free mice had little CSA in their sera as compared to conventionally housed mice. Similar activity has been found in the serum and urine of patients with acute or chronic leukemia or infectious mononucleosis, and in low concentration in normal persons. Partial characterization of CSA from several sources (mouse serum, human urine, mouse embryo cells) revealed similar molecular weights (45 to 100,000<sup>345,368</sup>), resistance to heat at 60°C for 30 minutes, and no loss of activity on dialysis. In mouse embryo extracts an inhibitor could be removed by dialysis, thus increasing CSA activity. Mouse CSA has shown some specificity in that marrow, spleen, fetal liver, and blood cells were stimulated to produce granulocyte colonies, but other tissue cells were not, nor were rat marrow cells stimulated. The polymorphonuclear neutrophils of man appear to secrete CSA and provide a better stimulus for human marrow colony growth than does urinary CSA. Blood leukocytes from patients with acute myeloblastic leukemia had little CSA activity.<sup>360</sup> In other studies, macrophages or monocytes were shown to release CSA whereas neutrophils were inhibitory.<sup>346a,328</sup> CSA was thought to act on the committed stem cell rather than on the more primitive CFU cell or later forms<sup>357</sup> (Fig. 6-8). Fluctuations in CSA content in animal or human serum and urine suggest that CSA has physiologic importance,<sup>347,361</sup> but this has not been clearly established as yet since CSA studies have been conducted almost entirely with the *in vitro* assay.

Still another granulocytopoiesis-stimulating factor has been described. This factor is active *in vivo*, diffuses into millipore cham-

bers to stimulate myeloid proliferation of blasts and promyelocytes, and is present only during the neutropenic and recovery phase which follows x-irradiation or vinblastine injection.<sup>364</sup> Preliminary studies suggest that its activity is not the same as that of CSA.

It is not yet clear how many of the factors described above represent separate and distinct control mechanisms of neutrophil production and release and which of them may be the same or closely related substances studied and assayed under different conditions and in different species.

### *Phagocytosis and Particle Ingestion*

The major function of neutrophilic granulocytes is to prevent or retard the intrusion of infectious agents and other foreign material into the host environment. This is accomplished by phagocytosis and digestion of the material. Neutrophils also release various substances into their environment and thus may have a secretory function as well.

*Endocytosis* is the process by which material is taken into a cell enclosed within pieces of plasma membrane without the material at any time occurring free in the cytoplasm of the cell (Fig. 6-10A).<sup>423</sup> Endocytosis is further divided into *pinocytosis* (drinking by cells) and *phagocytosis* (eating by cells). Phagocytosis can usually be seen by light microscopy while pinocytosis cannot because it involves ingestion of very small particles, such as macromolecules. Both processes involve invagination of the cell membrane and the formation of vesicles or vacuoles (phagosomes). Most cells appear capable of pinocytosis, but phagocytosis is a prominent activity of neutrophils, monocytes, macrophages, and, to a lesser extent, eosinophils and basophils. Like macrophages (page 272), polymorphonuclear neutrophils ingest foreign material (bacteria and a variety of particles), but they rarely ingest autologous cells<sup>462</sup>; similar particle selectivity appears to occur *in vivo*.<sup>102</sup>

Neutrophils and macrophages are motile and thus are free to migrate into sites of inflammation, apparently in a purposeful manner. Once in the area of inflammation

they come in contact with the foreign material, engulf it, and subject it to the bactericidal and digestive enzymes that they contain. This was first appreciated by Metchnikoff in the 1880's<sup>448</sup> when he observed the migration of phagocytes into areas of tissue damage in sponges and lower animals. From later studies he concluded that all resistance to infection resulted from the activities of phagocytic cells. However, work begun at about the same time demonstrated antibacterial activity in serum,<sup>412</sup> and by the early 1900's the concept that opsonins in the serum coat bacteria and render them easily ingested by phagocytes<sup>467</sup> became popular. The phagocytic cell then came to be regarded as only passively involved in resistance to infection. This view was strengthened when it was shown<sup>464</sup> that the enhanced phagocytosis of pneumococci previously exposed to serum resulted from the coating of the bacteria with antibody and complement. For many years phagocytosis was thought to depend on cell surface tension or charge effects<sup>404,431,450</sup> and to involve no expenditure of energy. This view was maintained even though it had been noted in 1933 that, during the phagocytosis of bacteria, leukocytes display an extra burst of oxygen consumption.<sup>400</sup>

The importance of the phagocytic cell itself to body defense was not reemphasized until the 1940's and later when Wood<sup>466</sup> showed that the outcome of infection was decided long before specific antibodies appeared in the serum. He demonstrated the ability of neutrophils to trap and ingest organisms in the absence of antibodies and serum factors and called the process "surface phagocytosis." It has since been shown that phagocytosis proceeds as well in a pure nitrogen atmosphere as in oxygen, that it is not inhibited by cyanide or dinitrophenol, and that inhibitors of glycolysis do inhibit phagocytosis.<sup>441,442</sup> It also has been found that a variety of enzymes and bactericidal activities reside in the neutrophil cytoplasmic granules and that these are discharged on phagocytosis.<sup>415,419,437</sup> Since the 1940's an understanding of chemotaxis, particle recognition and adsorption, opsonin activity, endocytosis, bacterial killing and digestion, and the proc-

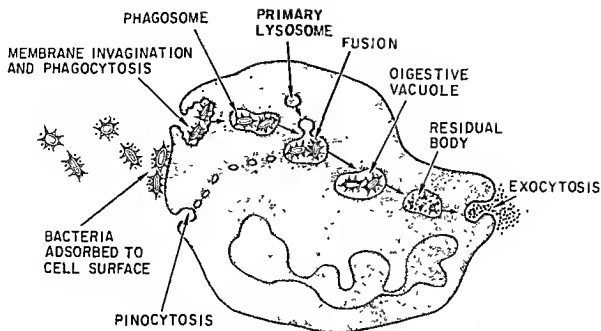


Fig. 6-10A Schematic diagram of endocytosis, both phagocytosis of immunoglobulin-coated bacteria and pinocytosis are shown. The fusion of primary lysosome with the phagosome to form the digestive vacuole, the subsequent degradation of the bacteria leading to the formation of a residual body, and the expulsion of indigestible components are also depicted.

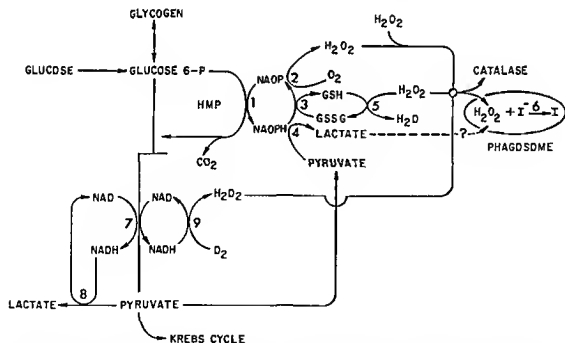


Fig. 6-10B Leukocyte metabolism as related to endocytosis and bacterial killing. 1, Glucose-6-phosphate dehydrogenase and 6 phosphogluconate dehydrogenase 2, NADPH oxidase 3, Glutathione reductase 4, NADPH linked lactic dehydrogenase 5, Glutathione peroxidase 6, Myeloperoxidase (MPO) 7, Phosphoglyceraldehyde dehydrogenase 8, NADH-linked lactic dehydrogenase 9, NADH oxidase (Modified from Klebanoff <sup>505</sup>)

esses associated therewith has accumulated with overwhelming rapidity. After years of controversy over the relative importance of these several factors it now appears that all are involved.<sup>468</sup>

### Chemotaxis<sup>435,447,458</sup>

Although the mobility of neutrophils and their concentration in inflammatory lesions were appreciated in the earliest experiments, the development of a two-compartment chamber with a membrane permeable to leukocytes separating the compartments has permitted easy quantitation of chemotaxis *in vitro* and has greatly facilitated investigation of chemotactic factors.<sup>401,405</sup> From such studies it is evident that both neutrophils and mononuclear phagocytes show directional migration under the influence of chemotactic agents, but a concentration gradient is needed for migration to occur. In the absence of a gradient, but with chemotactic factor present, random migration is enhanced and localization or "trapping" of the phagocytes occurs. Chemotaxis appears to be mediated by two means: *cytotaxins* which have a direct action on phagocytes (eg, casein, certain bacterial filtrates, and low molecular weight split-products of the complement components C5 and possibly C3, and a complex of C5, 6, 7) and *cytotaxigens* which are not chemotactic themselves but which generate chemotactic factor after interacting with serum, plasma, or components of complement. Examples of cytotaxigens are antigen-antibody complexes, endotoxin, certain bacteria, certain enzymes reacting with complement components, and lysosomes from neutrophils, macrophages, or liver. Certain cytotaxins appear to be specific for neutrophils (eg, culture filtrates of *E. coli*) while others such as casein induce migration of both neutrophils and monocytes.<sup>531</sup> In general, neutrophils are attracted by low molecular weight components.<sup>458</sup> The vasoactive amines, bradykinin, serotonin, and histamine have no chemotactic activity in the *in vitro* chamber.<sup>458</sup>

As yet only a few studies reveal the operation of chemotactic factors *in vivo*. In rabbit-

ear chamber experiments, random movement of leukocytes was seen in the center of injury, but preceding this a directional movement of leukocytes into the area was demonstrated.<sup>410</sup> The random movement is now suggested as reflecting the "trapping" of leukocytes in an area containing chemotactic factor but with no concentration gradient to provide direction.<sup>458</sup> In other studies a correlation between *in vitro* chemotactic activity generated by incubating minced rat tissue with serum and the *in vivo* accumulation of cells after injection of the activity was established.<sup>439</sup> However, a wide gap still exists between the significant role in inflammation that chemotaxis is recognized as playing and understanding of the mechanism by which it acts. The nature of receptors on the phagocytes is only beginning to be unraveled and we are ignorant of how, once recognized, the message is translated into directional locomotion.

### Particle Recognition and Adsorption

How phagocytes distinguish foreign particles and damaged autologous cells from normal self-components remains a mystery, but this capacity obviously is the essence of phagocytic function. Dog neutrophils have been shown to bind a specific type of IgG which enhances their phagocytic capacity for staphylococci in the absence of complement.<sup>432</sup> In other studies an alpha-1-glycoprotein capable of enhancing phagocytosis in the absence of complement was found in germ-free rat and guinea pig serum.<sup>426</sup> The presence of this activity in animals without prior exposure to foreign antigens suggests that it is not antibody and may be related to recognition and adsorption of foreign materials. However, how such factors enhance adsorption and phagocytosis is not clear.

It has been shown that neutrophils from guinea pig and sheep require divalent cations ( $\text{Ca}^{++}$  or  $\text{Mg}^{++}$ ) for adsorption and ingestion of particles,<sup>427</sup> and human leukocytes may require cations for attachment to a charged surface.<sup>419,409</sup> Presumably, in the absence of divalent cations, repulsive forces at the surfaces of the bacteria and the phagocytic cells

are sufficient to allow only a weak interaction.<sup>427</sup> In the absence of firm adsorption, bacteria are not ingested and can be washed off the cells.<sup>428</sup> However, from studies with macrophages it seems that divalent cations are needed not for particle adsorption but for the ingestion stage.<sup>432</sup> The osmolality of the medium is important also and high concentrations of glucose or salts may impair phagocytosis and leukocyte aggregation.<sup>413,444</sup> In certain tissues, such as the renal medulla, such factors may interfere with efficient phagocytosis.

*Opsonins*, long recognized as enhancing phagocytosis, act primarily on the particles rather than on the phagocytic cells, but the term has been applied in various ways since it was first used.<sup>406,452</sup> Opsonins include specific antibodies (heat stable), complement (heat labile), non-complement thermolabile factors, and perhaps other factors in the serum, such as lysozyme, which when adsorbed onto particles render them attractive to phagocytic cells. Clearer understanding of opsonin action is beginning to emerge as a result of the availability of procedures for fractionating immunoglobulins and complement into their several components. For example, in a study in rabbits of the *opsonin activity of antibodies* to *Proteus mirabilis* the IgG component was shown to enhance the uptake of living *Proteus* organisms (in the absence of complement) while the IgM fraction had little effect.<sup>456</sup> In other studies, in the guinea pig, 7S antibody was fractionated into two components,  $\gamma_1$  and  $\gamma_2$ . The  $\gamma_2$  antibodies attached to macrophages but the  $\gamma_1$  fraction did not, and neither fraction became attached to polymorphonuclear cells.<sup>403</sup>

Thus only certain immunoglobulin fractions appear to have cytophilic properties and these may be specific for only one cell type. The portion of the antibody that attached to the cell was on the Fc fragment and thus on the H chains of the molecule. In addition, there is evidence that *Staphylococcus aureus* contains a protein A substance which binds nonspecifically to the Fc portion of IgG and by this means blocks phagocytosis.<sup>425</sup> All of these investigations suggest that the Fab por-

tion of antibody reacts with organisms while the Fc portion contains cytophilic-binding sites. Thus immunoglobulins appear to facilitate contact between particles and those phagocytes with the necessary receptor sites. At present there is good evidence for such receptor sites on macrophages and polymorphonuclear cells.<sup>710</sup>

The opsonic activity of complement and other heat labile components of serum appears to be even more complex and is less well understood,<sup>406</sup> but the point to be made is that there are substances in normal serum that probably play an important role in promoting phagocytosis in the absence of specific antibody. Advancement in our understanding of foreign particle recognition and adsorption awaits elucidation of cell membrane structure, receptor sites thereon, and interactions with serum proteins, antibodies, and particle surfaces.

### Ingestion and Degranulation

The ingestion of particles by phagocytic cells requires the expenditure of energy<sup>441</sup> and is accompanied or immediately followed by a variety of complex biochemical events.<sup>460</sup> Although neutrophils contain a cytochrome system<sup>415</sup> and may use both aerobic and anaerobic mechanisms to produce energy, they utilize anaerobic glycolysis to support phagocytosis.<sup>454</sup> During ingestion the particle is surrounded by pseudopodia and the cell membrane invaginates to enclose and internalize it.<sup>453,494</sup> This is rapidly followed by a fusion of lysosome granules with the phagosome and the release of lysosomal contents, antibacterial substances and digestive enzymes into the phagocytic vacuole<sup>419,437</sup> (Fig. 6-10A). It will be recalled that there are two and perhaps three distinct types of neutrophil granules with different enzyme content (page 228). Since, after extensive phagocytosis, lysosomal granules are essentially absent the several types must all participate in the degranulation process. No migration of lysosomes from distant parts of the cell toward the phagosome has been recognized, but in neutrophils the contents of

specific granules are released into the phagosome first (leukocyte alkaline phosphatase was detected in phagosomes within 30 seconds) while the primary granule contents were found in the phagosome later (after one to three minutes).<sup>399</sup> The mechanism of phagosome-lysosome fusion is unknown, but it has been proposed that the continued interaction of complement and antibody within the phagosome releases lysolecithin which then acts on the lysosomal membrane to break it down. Fusion of phagosome and lysosome ensues.<sup>406</sup> The fragility of lysosomes at acid pH may also facilitate fusion.<sup>418</sup> After fusion, bacteria are usually quickly killed and the phagocytosed material is digested (see below).

A number of metabolic changes have been observed to occur during phagocytosis: there is a modest increase in glycolysis and lactate production and the pH within phagocytic vacuoles falls<sup>440,510,519</sup>; a two- to threefold increase in oxygen consumption occurs (which is resistant to cyanide and therefore does not involve mitochondrial cytochrome oxidation)<sup>454</sup>; consumption of the number one carbon atom of <sup>14</sup>C-labeled glucose and its conversion to CO<sub>2</sub> via the hexose monophosphate shunt increases six- to sevenfold<sup>515</sup> (also resistant to CN and other metabolic inhibitors); NADPH oxidation is increased fivefold, NADH oxidation is increased thirtyfold (pH 7.0),<sup>513</sup> and the dye, nitroblue tetrazolium (NBT), is reduced to blue formazan apparently by NADH oxidase<sup>482</sup>; formate oxidation is increased apparently by a peroxidase interacting with the increased amount of H<sub>2</sub>O<sub>2</sub> formed<sup>501</sup>; there is increased synthesis of membrane lipids<sup>429,492</sup>; and chloride and iodide ions are oxidized apparently via myeloperoxidase catalysis of the hydrogen peroxide generated.<sup>506</sup> Some of these reactions are illustrated in Figure 6-10B.

Since most of these events occur in the first several minutes after particle ingestion, the relation of one process to the others has been difficult to unravel.<sup>412</sup> However, the significance of these metabolic changes has become clearer since it was shown that neutrophils from patients with chronic granulomatous

disease (CGD) (see page 1326) do not exhibit the burst of oxygen uptake or glucose utilization via the hexose monophosphate shunt that normally occurs with phagocytosis.<sup>482,499</sup> Since particle ingestion and degranulation appear to be normal in CGD<sup>483,498,502</sup> but bacterial killing is not,<sup>498</sup> it should be possible to separate the metabolic events associated with each phase by comparing events in normal and in CGD neutrophils. From such studies it appears that increases in glycolysis and membrane lipid synthesis<sup>492</sup> are integral parts of the ingestion-degranulation process, while the other changes are involved with bacterial killing<sup>442</sup> even though they begin almost immediately after particle ingestion and before extensive degranulation has occurred.

### *Bacterial Killing and Digestion*<sup>503</sup>

Since bacterial killing decreases under anaerobic conditions whereas phagocytosis does not, it is thought that the "respiratory burst," mentioned earlier, is related to bactericidal activity. The nature of the events that take place has been surmised as the result of extensive investigations of the pathogenesis of chronic granulomatous disease (Chapter 42).

### *Metabolic Products*

The metabolic products of neutrophil metabolism that may have bactericidal action are lactic acid and H<sub>2</sub>O<sub>2</sub>. After particle ingestion the intraphagosomal pH has been reported to decrease to between 3.0 and 6.5.<sup>410,510,519</sup> It is known that some organisms such as pneumococci are sensitive to an acid pH while others tolerate acid environments without damage. In addition to a possible direct bactericidal effect the acid environment of the phagocytic vacuole may enhance the activity of lysosomal hydrolytic enzymes, most of which have optimal activity at acid pH. H<sub>2</sub>O<sub>2</sub> and superoxide formed during phagocytosis may have a direct bactericidal effect<sup>481,501</sup> and may also mediate other reactions (see below). It has been shown that anaerobic

conditions, phenylbutazone, catalase,<sup>505</sup> and hydrocortisone<sup>511</sup> decrease  $H_2O_2$  production and inhibit microbicidal activity, whereas the addition of  $H_2O_2$  to an anaerobic system partially restores the bactericidal effect.

### Nonenzymatic Agents

The nonenzymatic agents with antimicrobial activity include phagocytin, leukin, a group of cationic proteins, and lactoferrin. *Phagocytin*<sup>495-496</sup> was isolated from rabbit heterophils and shown to have antimicrobial activity that was optimal at acid pH, was temperature dependent, and was consumed during bactericidal activity. Another substance, *leukin*, was also isolated from rabbit cells and was shown to kill gram-negative organisms especially well at acid pH.<sup>520</sup> It is not yet clear whether phagocytin and leukin are different substances, but the latter appears to be clearly different from lysozyme and antibacterial histones.<sup>520</sup>

Leukocyte granules also contain a group of *cationic proteins* with differing bactericidal properties.<sup>523</sup> Some fractions are highly active against streptococci or staphylococci<sup>544</sup> while others are active against gram-negative organisms.<sup>523</sup> In rabbit heterophils these proteins appear to be located in the primary and secondary granules,<sup>485</sup> and are released after phagocytosis. In abscesses induced in rabbits and guinea pigs the causative organisms are coated by cationic protein at the time of microbial killing.<sup>521</sup>

*Lactoferrin* is still another bactericidal protein found in the specific granules of rabbit heterophils<sup>486</sup> and in human neutrophils<sup>536</sup> as well as in many secretions (milk, mucus, etc) and exudates.<sup>513</sup> Its strong chelating properties appear to account for its bactericidal effects and, in contrast to transferrin, this property is maintained at the low pH values encountered in exudates.

### Enzymatic Antimicrobial Activity

Neutrophil lysosomes contain a number of enzymes (see pages 228, 230) but only two of these, lysozyme and myeloperoxidase, have been shown to have microbicidal activity.

*Lysozyme* is a basic protein present in both primary and secondary neutrophil granules and is capable of hydrolyzing the cell wall of certain bacteria, thus effecting their death. Although the number of organisms susceptible to the direct action of lysozyme is small, some are made sensitive to its action after exposure to antibody and complement or to  $H_2O_2$  and ascorbic acid.<sup>505</sup> Usually bacterial death appears to precede the action of lysozyme and thus its action may be mostly digestive. The leukocytes of Guernsey and Hereford cattle contain no lysozyme but kill organisms normally.<sup>517</sup>

*Myeloperoxidase* (MPO) is present in high concentration in the azurophilic, primary granules of neutrophils and is released into the phagosome during granule lysis.<sup>505</sup> MPO has bactericidal, fungicidal, and viricidal activity<sup>505</sup> which is thought to depend on the oxidation and fixation of chloride, bromide, or iodide, a process which is mediated by MPO and the hydrogen peroxide generated during phagocytosis.<sup>504,506</sup> In addition to halides, thyroxine or triiodothyronine may act as a cofactor in this reaction. It is known that leukocytes concentrate iodide, thyroxine, and triiodothyronine, and an accelerated turnover of the latter two compounds occurs during bacterial infection.<sup>505</sup> Since azide inhibition of MPO greatly decreases microbicidal activity of normal leukocytes, the MPO system appears to be an important defense mechanism.<sup>501</sup> However, in patients with MPO deficiency the activity of non-MPO-dependent systems appears to be increased, thus partially compensating for the MPO deficiency.<sup>504</sup> This may explain the finding of increased susceptibility to infection in only one of five MPO-deficient patients.<sup>508</sup>

Phagocytosis is not always successful in killing bacteria, however. Some organisms (eg, certain virulent staphylococci) may survive and multiply within neutrophils and appear to kill them, thus overcoming the defense mechanism.<sup>544</sup> Still other materials ingested by neutrophils, such as the uric acid crystals of gout or the hydroxy apatite crystals of pseudogout, may cause breakdown of the phagosome wall and release the hydro-

lytic enzymes into the cell sap.<sup>522</sup> This is fatal to the cell; it then lyses and releases its enzymes into the surrounding tissues where they cause tissue damage and secondary inflammation. In certain streptococcal and other infections, bacterial exotoxins (eg, streptolysin) are released and damage the phagosomal membrane, thus killing the cell in a similar manner<sup>523</sup>; the infecting organism is freed in the process. Also certain vitamins (vitamin A) and drugs are incorporated into phagosomal membranes and render them fragile and readily susceptible to rupture, thereby leading to inflammation.<sup>522</sup>

*Digestion of bacteria* is demonstrated both by changes in the morphologic appearance of phagocytosed organisms and by the release of labeled fragments of bacteria into the surrounding medium.<sup>489,523</sup> Digestion is thought to result from the action of the acid hydrolytic enzymes released into the phagosome from the primary lysosome. Metabolic blocking agents such as iodoacetate, cyanide, and arsenite, which inhibit glycolysis and respiration, had no effect on digestion once the bacteria were within the cell.<sup>490</sup> The rate of digestion was different for RNA, lipids, DNA and protein, and immune sera delayed the digestion of coated bacteria. Some bacteria ingested by neutrophils may be killed and digested slowly (eg, certain pneumococci), the undigested material remaining as myelin or residual bodies.

### Secretory Functions of the Neutrophil

In addition to the fact that the contents of the neutrophil are released passively, as a result of cell lysis, there is evidence that a variety of substances are actively secreted by leukocytes in vitro.<sup>548</sup> These substances have been shown to originate from the granule fraction and include ribonuclease, deoxyribonuclease, betagluconidase, hyaluronidase, phagocytin, lysozyme, and histamine.<sup>548</sup> Recently, vitamin B<sub>12</sub>-binding  $\alpha$ -globulin<sup>532</sup> and leukocyte pyrogen<sup>545</sup> have been added to the list of secretory products. Since some of these substances are present in plasma normally and the concentration increases in pa-

tients with diseases involving the neutrophil system,<sup>530,550</sup> it has been suggested that neutrophils may serve a secretory function in vivo as well as a phagocytic role.<sup>532,548</sup>

Best studied is the  $\alpha_1$ B<sub>12</sub>-binding protein, *transcobalamin I*.<sup>532,547</sup> This is thought to function as a storage protein for vitamin B<sub>12</sub> and is a poor source of metabolically available vitamin<sup>538</sup> (see also Chapter 4, page 139). Markedly elevated transcobalamin I levels are seen in chronic myelocytic leukemia and in myeloid metaplasia; low values occur in chronic leukopenia and aplastic anemia<sup>538</sup>, and good correlation with blood granulocyte pool size has been reported.<sup>530</sup> These findings support the hypothesis that transcobalamin I is a secretory product of neutrophils and that the amount produced is related to the total neutrophil mass.

Another substance present in both primary and secondary neutrophil granules is *lysozyme*; it is also present in monocytes, serum, and tears and other secretions.<sup>542</sup> Increased concentrations in serum and urine are found in association with monocytic and myeloblastic leukemias.<sup>542,550</sup> It has been proposed that lysozyme is released from neutrophils when they are destroyed and that serum lysozyme may provide a measure of granulocyte turnover rate.<sup>534</sup> However, reasoning by analogy with the B<sub>12</sub>-binding protein and from the lack of correlation of granulocyte turnover rate with serum lysozyme levels in neutropenic patients,<sup>550</sup> it seems at least as likely that lysozyme is produced by both neutrophils and monocytes and may be either secreted by the intact cells or is released from them when they are destroyed.

In an in vitro system, *endogenous pyrogen* is produced by a variety of mammalian neutrophils and monocytes, including those of man.<sup>549</sup> Pyrogen production is activated by the phagocytosis of bacteria or after exposing the cells to endotoxin, or, in the case of human cells, to etiocholanolone. The process is temperature dependent and appears to require transcription of messenger RNA and its translation by ribosomes into new protein synthesis.<sup>540</sup> Leukocyte pyrogen cannot be released from blood leukocytes unless they



have been activated and a three- to four-hour latent period has elapsed; the amount produced is related to the degree of phagocytosis.<sup>545</sup> Leukocyte pyrogen has been detected in the serum of animals after endotoxin injection and thus the secretory process appears to be operative *in vivo* as well as *in vitro*.

### Eosinophil Series—Kinetics, Properties, and Functions

The distinctive morphologic appearance of eosinophils and the fact that these cells tend to appear at sites of foreign protein and parasite deposition and in association with allergic reactions have resulted in an enormous body of literature. However, understanding of the production, turnover, and function of eosinophils is limited. This is due in part to the fact that eosinophils constitute only a small proportion of marrow and blood leukocytes in normal man (Tables 2-1 and 6-4) and animals and thus quantitative studies are difficult to carry out. The available data are limited largely to studies in laboratory animals.<sup>601,603,611,651,676,677</sup>

The production of eosinophils, as well as that of neutrophils, occurs only in the bone marrow in normal man. Some eosinophil production has been reported to occur in the spleen of rodents,<sup>611,642</sup> but it now seems that only maturation occurs there.<sup>677</sup> Marrow eosinophilic promyelocytes and myelocytes are capable of mitosis (mitotic index < 2%)<sup>601,676</sup>; the metamyelocytes and more mature forms are usually regarded as post-mitotic stages undergoing maturation.<sup>611,651</sup> The flow of cells through the system is considered to follow a pipeline pattern as with neutrophils and there is a substantial marrow reserve of mature cells which can be mobilized on demand.<sup>611,642,651,676,677</sup>

#### *Production and Turnover in the Bone Marrow*

After the parenteral injection of tritiated thymidine into rodents, only the promyelocyte and myelocyte eosinophils were labeled initially.<sup>627,676</sup> The labeling indices appeared

to be somewhat higher than in the neutrophil series and label appeared in nondividing marrow eosinophils within four hours.<sup>627</sup> The mean myelocyte turnover time in rats has been estimated to be 40 hours to three days<sup>620,627</sup> and the total marrow transit time is reported as 5.5 days.<sup>676</sup> The minimal transit time from labeling with DNA precursors to appearance of labeled cells in the blood, the *emergence time*, has varied somewhat from species to species but is about 36 hours in mice,<sup>603,622,640</sup> 24 to 67 hours in rats,<sup>603,604,620,627,642,677</sup> and 60 hours in guinea pigs. When comparisons between neutrophils and eosinophils have been made the emergence time for eosinophils has been 20 to 24 hours shorter than that for neutrophils.<sup>622,640</sup> The mean transit time from myelocyte labeling in the marrow to peak radioactivity in the blood was about three to four days in animals. Detailed measurements of cell cycle kinetics for the eosinophil series in the rat have been reported, as well as measurements of eosinophil marrow mass.<sup>601</sup> In the guinea pig, it was estimated that there are 300 to 400 marrow eosinophils for each eosinophil in the blood with relative compartment sizes as follows: early forms, 50; metamyelocytes, 50; band and segmented forms in the bone marrow, 300; and circulating eosinophils, 1.<sup>651</sup> In the rat, in one study<sup>676</sup> only 10% of eosinophils were mature forms, while, in another,<sup>601</sup> two thirds of marrow eosinophils were nondividing.

In the only data for man, the maturation and emergence time of eosinophils was shorter than that for neutrophils, and DNA synthesis time was about the same as for neutrophils.<sup>679</sup> Kinetic studies in patients with eosinophilia suggest a pattern similar to that of neutrophils with a three- to four-day emergence time and a nine-day mean transit time.<sup>649</sup>

#### *Eosinophil Kinetics in the Blood*

In animals, the blood turnover time has been estimated to be less than 24 to 35 hours.<sup>603,620</sup> After continuous infusion of <sup>3</sup>HTdR into rats, heavily labeled eosinophils

left the blood to enter the tissues in a random fashion with a  $t_{1/2}$  of 8 to 12 hours.<sup>612</sup> After a single pulse label, a  $t_{1/2}$  of 6.7 hours and exponential disappearance were observed.<sup>617</sup> In man, random disappearance of blood eosinophils has also been reported,<sup>679</sup> and, in one patient with eosinophilia, radiochromate-labeled eosinophils left the blood exponentially with a  $t_{1/2}$  of about five hours.<sup>649</sup> It has been suggested that eosinophils may return to the blood,<sup>649</sup> but the evidence for this is not very convincing.

The fate of eosinophils is no better understood than is that of the neutrophil. Eosinophils are found in saliva in man<sup>679</sup>; in rodents, large numbers are present in the gastrointestinal tract in which site they survive less than six days.<sup>642</sup>

#### Effect of Adrenal Steroids and ACTH on Eosinophil Kinetics<sup>604,609,650</sup>

Adrenal steroids and ACTH produce eosinopenia in man<sup>681</sup> and suppress foreign protein-induced eosinophilia in animals.<sup>609,650</sup> The means whereby these effects are accomplished are uncertain. Lysis of eosinophils would not seem to be a satisfactory explanation since adrenal steroids do not lyse eosinophils *in vitro*<sup>609</sup> or in the bone marrow or tissues of animals given steroids.<sup>604,616</sup> Steroid eosinopenia occurs within two to three hours; consequently cessation of marrow eosinophil release as the sole explanation does not seem plausible if the estimates of blood eosinophil survival time given above (5 to 24 hours) are approximately correct. Isotopic studies suggest that a single injection of steroid produces reversible sequestration of eosinophils, presumably in the reticuloendothelial system,<sup>604,616</sup> while continuous steroid administration appears to interfere with marrow eosinophil release.<sup>604</sup> No mechanism for acute sequestration has been offered.<sup>611,651</sup> Since the blood eosinophil concentration increases after chronic infusions of histamine<sup>610</sup> or after the administration of histamine-releasing drugs,<sup>707</sup> and since histamine is reported to be chemotactic for eosinophils,<sup>610</sup> it has been suggested

that histamine may normally stimulate eosinophil release and/or production.<sup>609,611,650,651</sup> Chronic steroid administration might then affect the system by suppressing histamine release perhaps by stabilizing basophil and mast cell lysosomal membranes, by interfering with histamine biogenesis,<sup>647</sup> or by still other poorly defined mechanisms.<sup>650</sup>

#### Control Mechanisms

Since bacterial infections and certain other inflammatory events induce neutrophilia and, usually, eosinopenia, while antigen-antibody reactions and certain foreign proteins cause eosinophilia, separate control mechanisms for the two cell systems are suggested. This concept is supported by reports of an eosinophil-releasing factor<sup>677</sup> and a diffusible factor capable of stimulating eosinophil production by blasts and promyelocytes in millipore chambers implanted in the peritoneal cavity of mice.<sup>653</sup>

#### Normal Values and Physiologic Variations

In man, the normal concentration of eosinophils in the blood is less than  $0.7 \times 10^9$  cells/l (Table 6-4) and the mean value is  $0.15 \times 10^9$  cells/l as determined from the total leukocyte count (electronic counter) and the differential count. Direct measurements of absolute numbers of eosinophils<sup>611,674</sup> gave very similar normal values in some studies (mean  $0.159 \times 10^9$  cells/l, limits 0.054 to  $0.465^{615,682}$ ), but somewhat lower figures (mean values less than  $0.05 \times 10^9$  cells/l and upper limits less than about  $0.11 \times 10^9$ /l) in others.<sup>656</sup> Diurnal variation in the eosinophil count with high values late at night and low values at noon have been reported by some workers,<sup>615,639,682</sup> but others found no pattern common to all subjects.<sup>600</sup> Emotional stress may be associated with a decrease in total eosinophil concentration,<sup>653</sup> while exercise produces a transient increase.<sup>637</sup> Fluctuation in eosinophil count during the menstrual cycle has been reported, but the results have been variable and discrepant.<sup>637,665</sup>

### Properties and Functions

That eosinophils take part in the response to foreign protein has been recognized since the institution of antiserum therapy in the early 1900's and the production soon thereafter of allergic reactions in animals. It has also been evident that the eosinophilic response occurs in previously sensitized animals as a result of reactions associated with the interaction of antigen and antibody.<sup>659</sup> The search for substances chemotactic for eosinophils has been pursued extensively in the hope that some understanding of eosinophil function could be derived therefrom. With immunofluorescent labeling methods it was demonstrated that injected soluble or insoluble antigen-antibody complexes attract eosinophils and are phagocytized by them,<sup>663</sup> but excess antigen or antibody does not produce these effects.<sup>660</sup> A localized eosinophilia develops within 24 hours of the application of pollen extract to pollen-sensitive individuals but does not occur in normal subjects.<sup>644</sup> This reaction can be diminished by a series of desensitizing injections, presumably as a result of the formation of new incomplete or blocking antibodies which may interfere with the interaction of antigen and receptor sites on sensitized cells.<sup>646</sup> Other studies in which several types of antigen-antibody complexes or protein aggregates were injected into animals suggest that eosinophils may be attracted to substances with a particular molecular configuration.<sup>632,633</sup> A variety of other materials, many of them proteolytic enzymes, and even inert materials such as asbestos have induced eosinophilia when injected intraperitoneally into mice<sup>623</sup>; it has been suggested that they do so by activating the coagulation mechanism to produce fibrin.<sup>667</sup> It has been demonstrated that fibrin or proteolytic enzymes that promote fibrin production are chemotactic for eosinophils and that eosinophils contain profibrinolysin in their granules.<sup>667</sup> There also is evidence that basophils and tissue mast cells from sensitized subjects release their histamine when exposed to the sensitizing antigen.<sup>670</sup> The reported eosinophilotactic prop-

erties of histamine were mentioned above; it has been suggested that one function of eosinophils is to destroy histamine.<sup>611,651</sup> An extract of eosinophils has been shown to reduce the edema produced by the injection of histamine, 5-hydroxytryptamine, or bradykinin and it appears that an important function of eosinophils is to limit the effects of some of the biochemical mediators.<sup>611</sup>

Since all of the above-mentioned studies involved intact animals it is impossible to evaluate adequately the chemotactic properties of the substances employed. However, the demonstration that sensitized lymphocytes interact with appropriate antigen and produce a variety of soluble substances, such as migration inhibition factor, cytotoxins, and chemotactic factors,<sup>635</sup> suggests that the mobilization of eosinophils may be mediated by similar indirect means.<sup>618</sup> It has been shown that the reaction of immune complexes with the complement system produces a trimolecular complex,  $C_5,6,7$ , and several other substances that are chemotactic for both eosinophils and neutrophils.<sup>684</sup> These substances include soluble factors in bacterial culture filtrates and reaction products of serum and either immune complexes or plasminogen.<sup>684</sup> In addition, a substance selectively chemotactic for eosinophils has been found.<sup>628</sup> Only complexes that can fix complement (IgG or IgM, but probably not IgA or IgE) appear capable of generating chemotactic factor.<sup>657</sup> The fact that IgG<sub>1</sub> mediates the eosinophil-rich anaphylactic reaction while IgG<sub>2</sub> mediates the neutrophil-rich Arthus reaction<sup>661</sup> suggests that different cell receptor sites may play a role in chemotaxis and in phagocytosis of opsonized particles.

In addition to observations of eosinophil reactions after a second exposure to antigen, as discussed above, there is evidence that eosinophils may be involved very early in the immune response; they are phagocytic for antigen<sup>639</sup> and have been shown to accumulate in local lymph nodes within several hours after injection of antigen<sup>632,633</sup> and before antibody could be present.

No clear secretory function of eosinophils has been established. *Charcot-Leyden* crystals

form at sites of eosinophil or basophil concentration. These appear to be protein in nature and come from the cell cytoplasm rather than the contents of the granules.<sup>606</sup> This crystal formation does not seem to be the result of secretory phenomena.

The enzymes contained in eosinophil granules are similar to those in neutrophils except that eosinophils have a high content of peroxidase and aryl sulfatase and contain no lysozyme or phagocytin.<sup>607</sup> The absence of these antimicrobial substances has been taken to indicate that bacterial killing is not a major function of eosinophils.<sup>607</sup> However, a comparison of phagocytosis by eosinophils and neutrophils from human subjects demonstrates essentially similar processes in the two cell forms.<sup>625</sup> Eosinophils ingest bacteria, polystyrene particles, or fungi, but a smaller percentage of eosinophils than neutrophils participates and the eosinophils do so less avidly than do the neutrophils; eosinophils did not ingest antibody-coated red cells. Degranulation occurs<sup>607,625</sup> and the metabolic events associated with phagocytosis are similar to those observed in neutrophils.<sup>625</sup> However, more pronounced production of peroxide has been found in eosinophils than in neutrophils, apparently as the result of the high concentration of NADPH oxidase in eosinophil granules. In contrast, neutrophils derive  $H_2O_2$  through cytoplasmic NADH. Nevertheless, in spite of the enhanced peroxide generation, eosinophils were less effective in killing bacteria than neutrophils.<sup>613</sup>

#### Basophil Series—Kinetics, Properties, and Functions

The physiology, kinetics, and functions of basophils, the least numerous of the blood leukocytes in man, are poorly understood. Soon after their description by Ehrlich in 1891, basophils were recognized as probably being different from the tissue mast cells, which had been described some 14 years earlier, in spite of their obviously similar staining characteristics.<sup>718</sup> The basophil is smaller and usually less granular than the mast cell, and it has a bi- or tri-lobed nucleus

while the mast cell is mononuclear. Other morphologic and biochemical differences and similarities are cited on pages 232, 233.

#### Kinetics

Basophils are thought to be produced in the bone marrow in a manner similar to that of the production of neutrophils and eosinophils.<sup>724</sup> When tritiated thymidine was given to human subjects the initial labeling indices for eosinophilic and basophilic myelocytes in the bone marrow were similar to those for neutrophilic myelocytes.<sup>721</sup> The kinetics of eosinophils and of basophils in the marrow also were similar to one another and to those of the neutrophil system; there was a three-hour lag period after tritiated thymidine injection before label was seen in metamyelocytes; a one-day lag period before labeled band forms were seen; and a 1.5-day period before label was present in segmented basophils in the bone marrow. The "emergence time" from tritiated thymidine injection to the appearance of labeled basophils in the blood was 2.5 days and peak values for percent labeled basophils (about 23%) were reached on day 7.<sup>721</sup> The sequential flow of label through the several stages of maturation in the marrow and into the blood appeared entirely compatible with the pipeline scheme already described for neutrophils (page 244, Fig. 6-8).

Because of the paucity of basophils in the blood, no studies of pool sizes, cell distribution, or half-disappearance time have been possible. As with other granulocytes the fate of basophils is unknown. Although they may migrate into the tissues, there is no evidence that they transform into tissue mast cells. In fact, *mast cells* have been thought to be derived from connective tissue cells, and the finding that less than 0.05% were labeled after tritiated thymidine injection into mice suggests that the mast cell population turns over very slowly; the estimated turnover time is 9 to 18 months.<sup>737</sup> Other studies demonstrated that, after degranulation *in vivo* with the drug, 48/80, rat mast cells quickly reappeared and their granules were heavily la-

beled with administered radiosulfate.<sup>738</sup> Although this would support the earlier concept that mast cells are derived from nongranular precursor cells fixed in the connective tissues and that they release their granules on appropriate provocation and can replenish them rapidly,<sup>707</sup> subsequent studies demonstrated that after tissue mast cell degranulation *in vivo*, the tritiated thymidine labeling index increased from a control level of 0.06% to a maximum value of 4.5% two days after degranulation.<sup>727</sup> These results were considered to indicate that the connective tissue mast cells, though usually a very stable cell population, can be stimulated to divide. An equally plausible explanation, however, is that new cells were directed into the system from some precursor pool, perhaps blood monocytes.<sup>732</sup> This suggestion is based on the finding that, in the rabbit, antimacrophage serum (but not antilymphocyte serum) produced mast cell degranulation and karyolysis; by the third day thereafter, mononuclear cells containing metachromatic ("mast cell") granules reappeared, and the same cells also contained granules which stained with naphthol AS-D chloroacetate esterase, a stain which is thought to be specific for granules present only in cells of the monocyte-macrophage system.<sup>712</sup>

### *Physiologic Variations*

The development of methods for direct enumeration of basophils<sup>720,731</sup> has made possible a number of studies of variations in the concentration of basophils in the blood. In newborn infants, basophils are relatively numerous but decrease to normal adult levels ( $0.042 \times 10^3$  cells/ $1 \pm 0.048$  [2 SD]<sup>726</sup>) by the fifth postpartum day.<sup>719</sup> Basophils have the same diurnal rhythm as eosinophils, the highest blood concentration occurring during the night and the lowest in the morning.<sup>703,722</sup> No fluctuation during the day is associated with meals or moderate exercise has been noted.<sup>726</sup> In women the blood basophil concentration may fluctuate during the menstrual cycle, highest values occurring at the onset of menstrual bleeding; a drop in

basophil count has been associated with ovulation in humans and rabbits.<sup>734</sup> In rabbits, basophils accumulate in the ovaries and fallopian tubes at the time of ovulation. It has also been shown that steroid hormones cause a parallel decrease in basophil and eosinophil concentration in the blood, and a common influence of the pituitary-adrenal system on both cell lines has been postulated.

### *Properties and Functions*

The blood basophils have been shown to be capable of sluggish motility,<sup>4</sup> they have been noted to migrate into the skin or peritoneum after injection of foreign protein,<sup>740</sup> and they accumulate in skin windows in sensitized individuals.<sup>740</sup> Although basophils are capable of phagocytosis<sup>728</sup> it is not clear that this is their major function. Basophils and tissue mast cells also release their granule contents outside the cell (exocytosis) after exposure to a variety of stimuli such as mechanical irritation and, in sensitized subjects, to antigen<sup>701,715,740</sup> or cold.<sup>718</sup> Thus they appear to have a major secretory function.

The high content of histamine in basophils as compared with other blood leukocytes has been demonstrated repeatedly.<sup>708,728,735</sup> The histamine is contained within the basophil granules in amounts ranging from 1 to 2.4  $\mu\text{g}$  per  $10^6$  cells.<sup>708,728</sup> However, rat mast cells contain 40  $\mu\text{g}/10^6$  cells,<sup>728</sup> and since they are more numerous than basophils in the body, it is likely that only a minor fraction of total body histamine is present in basophils.<sup>728</sup> Human basophils contain histidine decarboxylase and thus can synthesize histamine.<sup>709</sup>

With anti-IgE antibody it has been shown that the Fc portion of IgE (reaginic antibody) is bound by specific receptor sites on human basophils.<sup>710,711</sup> The reaction of basophils with anti-IgE results in degranulation and the release of histamine *in vitro*, while injection into the skin produces erythematous wheal reactions.<sup>711,712</sup> Calcium and magnesium ions are necessary for degranulation and histamine release. A noncomplement component of serum, though not necessary for the reaction,

enhances it by increasing the amount of histamine released.<sup>711,712</sup> IgE is found on basophils of both normal and atopic individuals,<sup>711</sup> and contact with allergens results in degranulation.

Heparin also has been demonstrated in the granules of basophils and mast cells.<sup>700,709</sup> In addition to their involvement in hypersensitivity reactions, basophils appear to release heparin during postprandial lipemia,<sup>730</sup> and a correlation between the blood basophil concentration and plasma triglyceride level was reported.<sup>704</sup> It is believed that basophils facilitate triglyceride metabolism by releasing heparin, which in turn activates lipoprotein lipase.<sup>704</sup>

### Monocyte Series—The Mononuclear Phagocyte System—Kinetics, Properties, and Functions

#### Site of Production and Kinetics

Within the last decade the transfusion of allogeneic marrow cells into irradiated, recipient mice to produce chimeras has clearly established the bone marrow as the source of

monocytes and macrophages.<sup>92,108,133</sup> The transfusion of <sup>3</sup>HTdR-labeled, syngeneic marrow cells into irradiated rats has confirmed these findings,<sup>136</sup> and similar studies have shown that thoracic duct lymphocytes or cell suspensions from lymph nodes or thymus cannot serve as sources of macrophage precursors; spleen cells could, but they were not a major source.<sup>130,136</sup> After 24 hours of incubation with <sup>3</sup>HTdR, one third of marrow mononuclear phagocytes were labeled as compared with 2.2% in peritoneal macrophages and no uptake by blood monocytes.<sup>130</sup> These findings suggest that the marrow contains a proliferating population that supplies monocytes to the blood which in turn enter the tissues and become macrophages (Fig. 6-11).

#### Promonocytes

More detailed marrow incubation studies have revealed that a group of younger-appearing forms, larger in size than monocytes and with deeply basophilic cytoplasm, are heavily labeled after incubation with <sup>3</sup>HTdR as compared with no uptake of label

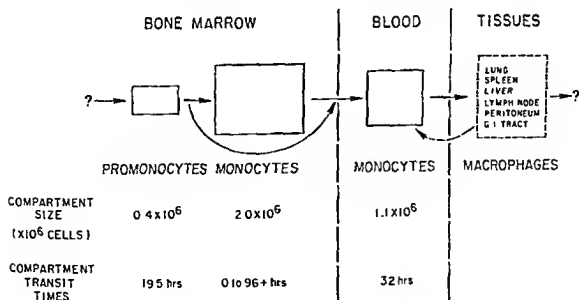


Fig. 6-11. Model of the production and kinetics of the monocyte-macrophage system in the mouse. The marrow and blood compartments are drawn to show their relative size. The compartment transit times are derived from <sup>3</sup>HTdR-labeling studies.<sup>177</sup> The curved, solid arrow emphasizes the rapid appearance of labeled monocytes in the blood after <sup>3</sup>HTdR injection and the apparent lack of a pipeline flow of cells through the system. The interrupted arrow denotes the possible return of avidly phagocytic macrophages from the tissues to the blood.

by typical marrow monocytes.<sup>777</sup> These younger, proliferating forms were termed "promonocytes." They were distinguished from monocytes and macrophages by hyperdiploid content of DNA in the majority,<sup>777</sup> and by morphologic and staining differences.<sup>778</sup> Promonocytes constitute about 15% of the mononuclear phagocytes in mouse marrow, but in human marrow only 2.9% of marrow cells were identified as promonocytes.<sup>759a</sup>

After the injection of <sup>3</sup>HTdR intravenously into mice, 70% of marrow promonocytes were labeled initially while marrow monocytes were essentially unlabeled. Subsequently this pattern changed. The promonocyte-labeling index remained almost constant during the next 24 hours while the marrow and blood monocyte-labeling indexes increased in an identical fashion.<sup>777</sup> The promonocyte grain count decreased while the grain count in marrow and blood monocytes remained constant at about one half that of the promonocytes during the first six hours of the study. Since monocytes do not divide, this suggests that they are derived from promonocytes after one division and that some rapidly enter the blood while others remain in the marrow (Fig. 6-11).

In normal man, only 12% of marrow promonocytes were labeled after incubation *in vitro* (<sup>3</sup>HTdR. Double-labeling studies *in vitro* (<sup>3</sup>HTdR and <sup>14</sup>CTdR) as well as serial <sup>3</sup>HTdR injections gave a mean DNA synthesis time of about 10 hours and a generation time of about 29 hours for promonocytes.<sup>759a</sup> From studies of blood monocyte labeling after intravenous injection of <sup>3</sup>HDFP the marrow proliferation pool transit time was estimated to be about 55 hours. This figure is about twice the monocyte generation time and suggests that there are two catenated promonocyte generations in man.<sup>759a</sup> After <sup>3</sup>HTdR injection, labeled monocytes appeared in the blood within five to seven hours, thus demonstrating an absence of a significant storage compartment.<sup>759a</sup>

A precursor of the promonocyte has not been identified; however, the ability of gran-

ulocytic colonies grown in agar or from the spleen to transform into macrophage colonies<sup>759,783</sup> suggests that early myeloid forms may differentiate along this as well as the granulocytic route (see also Chapter 2 and Fig. 2-6, page 52).

### Production in the Mitotic Compartment<sup>777</sup>

From the increase in promonocyte labeling index (LI) observed during studies in which <sup>3</sup>HTdR was injected every two hours into mice, the rate of promonocyte entry into DNA synthesis was found to be 5.15% per hour.<sup>777</sup> From this and the initial LI of 69.8% the average DNA synthetic time ( $t_s$ ) was calculated to be 13.6 hours ( $69.8/5.15 = 13.6$ ). The mean promonocyte generation time ( $t_g$ ) as calculated from  $t_s$  and LI ( $t_g = t_s/LI$ ) was 19.5 hours.<sup>777</sup> Very similar values for  $t_s$  and  $t_g$  have been reported for the rat.<sup>785</sup> From the size of the marrow promonocyte pool (see below) and  $t_g$  the daily production of monocytes can be calculated to be  $0.49 \times 10^6$  cells/day in mice.

The *promonocyte-monocyte population size of mouse marrow* as determined from measurements of total femur cellularity and differential cell counts was  $2.4 \times 10^6$  cells ( $0.4 \times 10^6$  promonocytes and  $2.0 \times 10^6$  monocytes). From this one might infer that there is a marrow monocyte reserve similar to the marrow neutrophil reserve. However, a comparison of the increase in percent of labeled monocytes in blood and marrow after <sup>3</sup>HTdR injection showed little delay (less than two hours in mice and less than eight hours in the rat) in the appearance of labeled monocytes in the blood.<sup>730,777,785</sup> These observations together with a marrow monocyte turnover rate of only 1.5% per hour suggest that monocytes do not mature and leave the marrow according to a pipeline pattern. Rather, some cells appear to leave the marrow soon after production (perhaps in a random fashion) while others may remain in the marrow for several days (Fig. 6-11).

Monocyte production can also be calculated from the turnover of blood monocytes

heavily labeled with  $^3\text{HTdR}$  during their formation. From the half-disappearance time of 22 hours<sup>130</sup> and a blood monocyte pool calculated (from the blood volume and absolute monocyte concentration) to be  $1 \times 10^6$  cells,<sup>177</sup> effective monocyte production in the mouse is calculated to be about  $0.3 \times 10^6$  cells/day.<sup>177</sup> This value is somewhat less than that determined from LI data (see above).

It has been estimated that in normal man the marrow promonocyte pool is about  $600 \times 10^6$  promonocytes per kilogram of body weight.<sup>759a</sup> From this, the labeling index and the DNA synthesis time (see above) the normal monocyte birth rate is calculated to be about  $7.0 \times 10^6$  monocytes/kg/day.<sup>759a</sup>

### Monocyte Kinetics in the Blood

The blood monocytes are a population of recently formed young cells on their way from the bone marrow to their ultimate sites of activity in the tissues. Labeled monocytes from the marrow enter the blood at a rate of 1.7%/hour in normal mice. The disappearance of heavily labeled cells from the blood follows an exponential curve with a  $t_{1/2}$  of 22 hours.<sup>130</sup> Somewhat longer  $t_{1/2}$  values (48 hours) have been reported in rats, and since the disappearance of label did not follow a single exponential it was suggested that the cells or the label recirculates.<sup>783</sup>

By labeling autologous blood with  $^3\text{HDFP}$ , reinjecting the labeled cells, and measuring the proportion of labeled cells present at later times by autoradiographic techniques, blood monocyte kinetics have been evaluated in man.<sup>759a</sup> In these studies the marginal monocyte pool was found to be about 3.5 times the size of the circulating pool. Blood monocytes left the vascular system in an exponential manner with a  $t_{1/2}$  of 8.4 hours (4.5 to 10 hours). From the blood pool size and the  $t_{1/2}$  the blood monocyte turnover rate was calculated to be about  $168 \times 10^6$  cells/kg per day. This figure is in very good agreement with the  $^3\text{HTdR}$  data described above.<sup>759a</sup>

Alterations in monocyte kinetics have been observed after the administration of adrenal

steroids to humans<sup>750</sup> and mice.<sup>775</sup> Profound monocytopenia develops promptly in both species and its duration and degree depend on the amount, solubility, and route of steroid administration. In man the cellularity of induced exudates was decreased by steroid administration.<sup>750</sup> In mice the number of cells harvested from the peritoneum was only moderately decreased (about 30%), but the flow of thymidine-labeled monocytes from the blood into the peritoneum in response to an inflammatory stimulus was markedly reduced. The mechanism of the sudden monocytopenia is not understood, nor is it clear whether the reduced cellularity at the site of inflammation merely reflects the monocytopenia or is the result of other steroid effects on the monocytes or the vascular wall or is due to yet other factors.

In septicemia there was enhanced monocytopenia in the marrow and the blood monocyte turnover rate was increased.<sup>759a</sup>

### Tissue Macrophages

The evidence that tissue macrophages are derived from blood monocytes which in turn come from marrow was cited above (page 267). After leaving the blood and entering various tissue sites, monocytes transform into macrophages and become avidly phagocytic.<sup>102,107,122,127,737</sup> Macrophages have traditionally been divided into "fixed" and "wandering" forms. The "fixed" macrophages take on a stellate or fusiform appearance as they stretch out along bundles of collagen or reticular fibers, and it may be difficult to distinguish them from fibroblasts by light microscopy. However, with the electron microscope, differentiation appears feasible and is made possible by the prominent rough endoplasmic reticulum of reticular cells, differences in mitochondria, and the presence of dense material (presumably tropocollagen), in contrast to the sparse rough endoplasmic reticulum and the prominent lysosome formation of fixed macrophages.<sup>770</sup> Some confusion has arisen because fibrocytes are phagocytic and take up vital dyes or metal particles, although to a consid-



erably lesser extent than do macrophages. However, fibroblasts do not come from monocytes; rather they appear to arise from connective tissue cells in the tissues surrounding an area of injury.<sup>770,771</sup> In sites such as connective tissue, spleen, liver, or lymph nodes, "fixed" macrophages are abundant and in normal tissues most macrophages appear to be of the sessile, inactive, fixed type. When stimulated by inflammation, some withdraw their dendritic processes and become actively motile, "free," or "wandering" macrophages, while others may enter DNA synthesis and divide or undergo other changes (see below). Thus classification as "fixed" and "wandering" macrophages appears to have little real functional significance except that the site of fixation may influence subsequent differentiation and function. For example, spleen or liver macrophages are exposed to the blood and appear to be responsible for clearing it of foreign materials and effete cells, while alveolar or lymph node macrophages, because of their locations, have somewhat different functions.

The number of tissue macrophages far exceeds the circulating monocyte pool, but a valid figure cannot be given. Estimates of the size of the macrophage pool in the spleen, liver, and bone marrow of rats suggest that there are about one billion cells in each location. Similar calculations indicate that in man there are 200 billion macrophages (RE cells) in the spleen, the liver, and the bone marrow (see Chapter 8, page 352). Thus there are at least 50 times as many macrophages in the spleen, liver, and bone marrow of man as there are monocytes in the blood.

Because macrophages in the various tissues are all derived from the same bone marrow precursors and migrate to their sites of function as blood monocytes, it has been proposed that this widely distributed cellular system be viewed as a single functional unit and termed the *mononuclear-phagocyte system*<sup>758</sup> (see Chapter 8). In preference to the designation "reticuloendothelial (RE) system" the new term emphasizes the common origin and closely related functions of the macrophages. It also avoids the implication inherent in the

term "RE system" that reticular cells (fibroblasts and fibrocytes) and endothelial cells form part of the system. These cells are specifically excluded since they have different origins and are much less phagocytic than are macrophages<sup>758</sup>; the latter fact was appreciated by Aschoff (see Chapter 8, page 351).

Although the macrophages in various tissues have common ancestors and carry out similar functions they may assume different properties, behave somewhat differently kinetically, and undergo specialized transformations in various body sites, as indicated below.

### Peritoneal Macrophages

Macrophages can be readily harvested from the peritoneum for study and are considered typical "free" macrophages. Their origin from bone marrow via blood monocytes has been mentioned (page 267). Studies in mice showed the free peritoneal cell population to consist of  $5$  to  $7 \times 10^6$  cells, about one third of which were macrophages.<sup>92</sup> When lethally irradiated mice were given homologous bone marrow, thus enabling them to survive, the peritoneal macrophage population was gradually replaced by donor cells over a five- to six-week period<sup>92</sup>; turnover time as estimated from <sup>3</sup>HTdR studies gave similar values, namely 20 to 40 days.<sup>130</sup> The fate of the replaced cells is unknown. Less than 3% of peritoneal cells took up <sup>3</sup>HTdR *in vitro*; thus they divide rarely in normal animals.<sup>130,135</sup> However, after injection of foreign substances such as glycogen, differentiation of peritoneal mononuclear cells into macrophages was accelerated, both as observed morphologically and as judged from increased acid phosphatase activity; concomitantly there was an increase in DNA synthesis (<sup>3</sup>HTdR uptake).<sup>757</sup> In mice infected with *Listeria monocytogenes*, marked transformation occurred within 48 hours.<sup>761</sup> This consisted of an increase in <sup>3</sup>HTdR uptake from < 2% initially to 14% at 48 hours, an increase in phagocytic and bactericidal ability, and increased spreading on glass surfaces. Since the enhanced phagocytosis and

spreading (ie, transformation into avidly phagocytic macrophages) were blocked by iodoacetate it was presumed that these changes reflected fundamental metabolic alterations that are important for enhanced, cell-mediated host resistance. A similar but slower response to BCG infection occurred.<sup>761</sup>

### Alveolar Macrophages

Early studies suggested that there are two populations of alveolar macrophages, one with a turnover time of seven days and another with a turnover of 35 days.<sup>751,752,773</sup> Studies in mouse chimeras seemed compatible with this in that two thirds to four fifths of alveolar macrophages appeared to come from donor cells while the remainder were of host origin.<sup>765</sup> However, other workers, using different methods to harvest the macrophages, showed all alveolar macrophages to be of donor origin.<sup>133</sup>

### Liver Macrophages (Kupffer Cells)

In normal mice given <sup>3</sup>HTdR, less than 1.5% of Kupffer cells were labeled within the first two hours, thus indicating a low rate of cell division and turnover.<sup>762</sup> With time the labeling index increased, reaching a maximum about 24 hours after the peak of blood monocyte labeling. From these data the Kupffer cells also appear to come from blood monocytes. The turnover time of Kupffer cells was estimated to be 60 hours in normal mice.<sup>129,776</sup> However, just as with peritoneal macrophages, the presence of infection with *Listeria* or BCG elicited a marked increase of <sup>3</sup>HTdR uptake and local proliferation of macrophages, followed by changes in macrophage properties and function.<sup>762</sup>

### Splenic Macrophages

These cells also have been shown to come from precursors in the marrow<sup>133</sup> and to undergo little proliferation under normal conditions (less than 2% <sup>3</sup>HTdR uptake).<sup>761</sup>

After appropriate stimulation (see Chapter 8, pages 352-354) or in the presence of infection<sup>761</sup> an increase in spleen macrophage proliferation occurs and the macrophages become more phagocytic and more effective in killing organisms.<sup>761</sup>

### Osteoclasts

Osteoclasts have been shown to form from fusion of tissue histiocytes,<sup>755,756</sup> and in the regenerating salamander limb have a life span of less than 10 days.<sup>755</sup> Osteoclasts differ from most macrophages in that they do not take up trypan blue, but like most macrophages they possess a high concentration of acid phosphatase activity.<sup>755</sup>

### Fate and Possible Recirculation of Tissue Macrophages

The low turnover and large mass of tissue macrophages in several body sites have been described, but the ultimate fate of these cells is unknown. Thus, although some alveolar macrophages are lost in the sputum, others must remain in situ for long periods as evidenced by the persistence of carbon-containing macrophages in the lung and regional lymph nodes.

Another unresolved question is whether macrophages reenter the blood and move from tissue to tissue. Early studies using vital dyes and carbon particles were interpreted as indicating that Kupffer cells and splenic macrophages could enter the blood and become alveolar macrophages,<sup>760</sup> but more recent studies have not confirmed this.<sup>766,767</sup> The fate of intravenously injected peritoneal or alveolar macrophages and Kupffer cells has been studied; Kupffer cells preferentially return to the liver while alveolar or splenic macrophages were found mainly in the spleen.<sup>766,767</sup> The presence of histiocytes in the blood in bacterial endocarditis and other severe infections suggests that either macrophages may reenter the blood from the tissues or transformation of monocytes to macrophages occurs in the blood.<sup>754</sup>

### *Normal Values and Physiologic Variations*

The variations in blood monocyte concentration with growth and development are shown in Figure 2-11 and normal values for the adult are given in Table 6-4. To our knowledge, nothing is known concerning physiologic variations in blood monocyte levels in normal man.

### *Control Mechanisms Regulating Monocyte Production*

Monocytopoietic substances probably exist, but information on this subject is as yet very meager. A macrophage growth factor derived from fibroblast cultures has been described,<sup>783</sup> but what role it may play, if any, in monocyte pathophysiology remains to be elucidated. Also a "monocytogenic hormone" has been found in the serum of rats after injection of complete Freund's adjuvant into lymph nodes. This activity produces striking blood monocytosis and is thought to increase monocyte precursor proliferation.<sup>789</sup>

### *Properties and Functions of Monocytes and Macrophages<sup>770,841</sup>*

As with the neutrophil, the blood monocyte is thought to be en route to its major sites of action in the tissues; there it *transforms into the macrophage*, which is the more active cell form. After migration into rabbit ear chambers, monocytes were observed to increase in size, the cytoplasm became more clear, and colorless refractile droplets that stained with neutral red developed at the periphery. The cells were now called "resting histiocytes" and exhibited little movement or activity. They did not divide in uninfamed tissue, but they did ingest red cells in their immediate vicinity although showing little inclination to migrate toward distant cells.<sup>102</sup> Blood monocytes contain small numbers of peroxidase-positive granules, but these are not seen in peritoneal or alveolar macrophages harvested from normal mice or after culture<sup>778</sup>; thus the granules apparently are

lost or used up in the transformation from monocyte to macrophage, and the presence of peroxidase-positive macrophages is thought to reflect the recent influx of blood monocytes.<sup>778</sup> In vitro,<sup>806,820</sup> as compared to monocytes, macrophages exhibit more active pinocytosis and phagocytosis, increase in glycolysis, cellular respiration and number of mitochondria, and enhanced enzyme activity<sup>806,811</sup>; active synthesis of lysosomal enzymes also has been demonstrated in macrophages.<sup>820</sup> The enzyme load of macrophages varies considerably, depending on their source, stage of activation, and other factors. Among other observations<sup>817,841</sup> the amount and rate of enzyme synthesis and the type of enzymes synthesized appear to be related to the nature of the material ingested by the cell.<sup>806,811</sup> Macrophages in the alveoli appear to be chronically stimulated as compared to those derived from other sites.

In short, the monocyte-macrophage is not an end-stage cell and thus its properties and functions are more varied and complex than those of other phagocytes. The macrophage survives much longer in the tissues than do neutrophils, is capable of cell division and transformation, and can be stimulated to synthesize a variety of enzymes and other substances, depending on the needs of the moment.<sup>841</sup>

### *Endocytosis*

A study of the in vitro phagocytic abilities of human blood monocytes demonstrated their ability to ingest a variety of bacteria, fungi, sensitized red cells, and polystyrene particles.<sup>812</sup> Monocytes were more energetic than neutrophils in ingesting polystyrene particles, fungi, and sensitized erythrocytes, and less effective in ingesting the bacteria studied (staphylococci and *E. coli*). Serum was not necessary for the uptake of polystyrene particles, but serum or IgG greatly enhanced the ingestion of bacteria and fungi. Monocytes ingested sensitized erythrocytes and this action was inhibited by certain subgroups of IgG<sup>801</sup> but not by others or by IgM or albumin.<sup>800,812</sup> Erythrophagocytosis

progressed poorly in suspension cultures although rosettes were plentiful; thus monocytes appeared to require surface contact for ingestion of these large particles. Neutrophils did not form rosettes or ingest sensitized erythrocytes, and neither neutrophils nor monocytes ingested unsensitized, in vitro-aged red cells, or antibody or endotoxin-damaged neutrophils. Glycolysis is required for phagocytosis by monocytes, but the associated metabolic changes are less striking than for the neutrophil and have been less well studied.<sup>806,812</sup>

Macrophages appear to be heavily involved not only in the phagocytosis of bacteria and effete or damaged autologous cells, but also in the uptake of a variety of macromolecules (pinocytosis). In vitro, the rate of pinocytosis was shown to increase with the concentration of serum in the medium and with the content of certain molecules therein, either natural polysaccharides, nucleotides, proteins (including antibodies), or synthetic macromolecules.<sup>808</sup> The pinocytotic vesicles were observed to fuse with preexisting secondary lysosomes (phagosomes) and Golgi-derived primary lysosomes, thus establishing a link between endocytosis and the changes (lysosome formation and enzyme synthesis) that occur with differentiation. Although the processes of pinocytosis and phagocytosis are similar, their metabolic requirements appear to be different in that pinocytosis is inhibited by anaerobic conditions or by inhibitors of respiration and oxidative phosphorylation.<sup>806,815</sup>

### Chemotaxis<sup>851</sup>

As with the neutrophil, monocytes or macrophages may come in contact with bacteria, effete cells, or other particles either by chance, as a result of chemotactic factors, or by virtue of antibody activity. It has been demonstrated that certain direct-acting chemotactic factors (cytotoxins from bacterial cultures) have greater attraction for neutrophils than for macrophages, while normal serum (not plasma) is more chemotactic for macrophages. Separation of normal serum on

Sephadex columns revealed that the macrophage chemotactic activity resided in a high molecular weight fraction (160,000 to 250,000 MW) while several fractions of lower molecular weight (5,000 to 35,000) were most attractive to neutrophils.<sup>851</sup> Plasma is not chemotactic for either cell type, but it has been shown that plasmin splits complement into neutrophil-active agents (C3<sub>a</sub> and C5<sub>a</sub>), and plasmin interaction with serum generates macrophage chemotactic activity. Thus the coagulation system appears important in the chemotactic attraction of monocytes and macrophages. There also is in vitro evidence that sensitized lymphocytes react with antigen and release factors chemotactic for macrophages.<sup>851</sup> As yet, macrophage chemotactic factors are less well understood than are factors attractive to neutrophils.

### Particle Recognition<sup>452,851</sup>

It has long been known that the ingestion of most particles by phagocytes is enhanced by factors in serum, specific antibody, complement, natural antibody, or perhaps still other factors. IgG antibodies facilitate attachment and uptake of erythrocytes by monocytes and macrophages.<sup>832</sup> There appear to be at least two different types of receptor sites for immunoglobulins on macrophages. The receptor site for 7S immunoglobulin does not require Ca<sup>++</sup> while 19S binding required Ca<sup>++</sup>. Both receptor sites are resistant to treatment with trypsin and thus are distinct from complement receptors.<sup>833</sup> Neutrophils and monocytes appear to have receptors for 7S but not for 19S immunoglobulin. Macrophages are able to bind certain antibodies (cytophilic antibody) and thus facilitate particle contact, but the role of this process in particle recognition is not yet understood. Some particles are ingested in the absence of serum recognition factors (eg, polystyrene, silica, glass) presumably because their surfaces are naturally attractive to phagocytes. Other particles resist attachment and appear to possess antiphagocytic properties, eg, the polysaccharide capsule of pneu-

mococci, the M protein and hyaluronic acid of streptococci, and the polyglutamic acid capsule of the anthrax bacillus. Bacteria or erythrocytes can be artificially opsonized by treating them with certain metals, tannic acid, or by other means that probably alter the particle surface. The activation of complement probably also results in alterations of particle surfaces and thus enhances adsorption and phagocytosis. A comparison of phagocytosis by insect hemocytes, "facultative" phagocytes such as tissue culture fibroblasts, and "professional" phagocytes (neutrophils, monocytes, and macrophages) has led to the suggestion that professional phagocytes differ from other cells in possessing receptor sites for immunoglobulins. It is proposed that this property serves to greatly enhance their ability to recognize and ingest foreign particles.<sup>845</sup> In the absence of such receptor sites the binding and uptake of particles by other tissue cells appear to depend on some crude means of recognition that identifies some basic property of the particle surface that is required for adsorption (perhaps affinity for aqueous or fatty substances or appropriate charge).

### Ingestion<sup>839</sup>

The mechanism by which a cell is triggered to ingest particles adsorbed to its surface is not known. It has been hypothesized that the cell recognizes a conformational change which occurs when antibody reacts with antigen,<sup>843</sup> but this does not explain the ingestion of inert particles (glass, polystyrene) in the absence of serum.<sup>839</sup> Studies of ingestion using polystyrene spheres of increasing size led to the suggestion that phagocytosis is triggered when a large enough surface area of the phagocyte reacts with the particle; however, this does not explain how a macrophage can adsorb to its surface several aldehyde-treated erythrocytes in balanced salt solution but will not ingest them unless opsonins also are available.<sup>839</sup> Perhaps aldehyde treatment alters the erythrocyte membrane enough to facilitate adsorption, but something more is needed to activate ingestion.

As with the neutrophil and monocyte, phagocytosis by macrophages requires *energy expenditure*. This is derived from anaerobic glycolysis.<sup>806,819</sup> An important exception is the alveolar macrophage which relies heavily on aerobic metabolism for its metabolic needs<sup>784,806</sup> and requires an oxygen tension greater than 25 mm Hg for maximal phagocytic activity.<sup>815</sup> Pinocytosis requires oxidative phosphorylation and is suppressed by inhibitors of respiration; thus it appears to be a somewhat different process than that of phagocytosis.<sup>819,845</sup> New protein formation also is required for continuing pinocytosis and the process is inhibited by puromycin or cycloheximide; presumably this reflects a requirement for new plasma-membrane formation.<sup>819</sup> Phagocytosis by the macrophage is accompanied by an increase in glycolysis, oxygen uptake, conversion of the 6 carbon of glucose to CO<sub>2</sub>, and other changes that also accompany phagocytosis by neutrophils and monocytes (see page 259). However, the increases are much less striking than those for neutrophils and are similar to those of the monocyte.<sup>784,806</sup> The relationship of these metabolic events to particle uptake, bacterial killing, and associated processes is even less clear than for the neutrophil.

The bactericidal properties of monocytes and macrophages are well documented since, within 20 minutes, susceptible ingested organisms are killed. However, very little is understood about how this is accomplished.<sup>806,811</sup> In contrast to neutrophils, monocytes and macrophages lack phagocytin, and cationic proteins and macrophages lack peroxidase. Both possess lysozyme and thus can split the B-1,4 glycosidic bond between N-acetylglucosamine and N-acetylmuramic acid in bacterial cell walls, but few organisms are sensitive to lysozyme in their native state. Some but not all monocytes deficient in myeloperoxidase have been shown to be deficient in fungicidal properties<sup>508</sup>; thus this enzyme must play some part in microbicidal action, but alternative mechanisms for bacterial killing appear to exist in its absence.<sup>504</sup> Once ingested, pinosomes or phagosomes move toward the nucleus and fuse with pri-

primary lysosomes or preexisting secondary lysosomes, thus being converted to secondary lysosomes (Fig. 6-10A). Colchicine may interfere with the movement of organelles that precedes fusion.<sup>837</sup> A significant difference from neutrophils is that monocytes and macrophages can synthesize new enzymes and replace expended lysosomes,<sup>818</sup> thus giving them more staying power in combating infection and inflammation.

Digestion of endocytized material proceeds in the acid environment of the secondary lysosome under the influence of the spectrum of enzymes present. Metabolic blockers do not inhibit digestion,<sup>490</sup> and only a marked reduction in ambient temperature will interfere.<sup>819</sup> Once started, digestion is rapid, over 80% of isotope from iodinated albumin, gammaglobulin, or hemoglobin being released from the cell into the medium in 20 hours. Labeled bacteria also are digested rapidly, but those coated with immune sera are degraded less rapidly than are uncoated organisms.<sup>490</sup> Digestion of sugars to molecules smaller than 200 molecular weight is accomplished before release from the lysosome; peptides are degraded at least to tripeptides before release.<sup>819</sup> Some materials are digested with difficulty or not at all and accumulate within the secondary lysosome<sup>819</sup>; in vitro examples are sucrose, ficoll, dextran sulfate, inulin, D-amino acid and di- and tripeptides.<sup>819</sup> In man the accumulation of indigestible materials in macrophages may lead to disease, as in the case of the lipoidoses (see Chapter 42), silicosis, and other granulomatous processes.

The synthesis and secretion of a variety of biologically active substances by cells of the monocyte-macrophage system are now fairly well established and include components of the complement system, transferrin, interferon, endogenous pyrogen, lysozyme, and colony stimulating factor.

Incorporation of <sup>14</sup>C-labeled amino acids into the third component of complement (C3 or  $\beta_{1c}$ ) and transferrin was accomplished by rat peritoneal exudate cells but not by thoracic duct lymphocytes.<sup>853</sup> In other species (mouse, rabbit, guinea pig, monkey, and

man), peritoneal and alveolar macrophages also produced B<sub>1</sub>C, but only mouse and rat cells produced transferrin. Human and monkey macrophages produced the C4 and C1<sub>q</sub> components of complement, perhaps C5 and C6 and also IgG. It should be noted that the synthesis of complement is accomplished by a variety of other cell lines as well, including tissue-culture fibroblasts, probably hepatocytes, and the guinea pig columnar epithelial cells of the intestine.<sup>853</sup>

Interferon production is a property of blood monocytes and peritoneal or alveolar macrophages. Polymorphonuclear neutrophils, spleen cells, and kidney cultures also produce interferon after viral infection, but macrophages produce larger amounts.<sup>853</sup> Exposure of cells to actinomycin inhibits interferon production. In vivo, in mice a major source of interferon production is the spleen as judged from drastic reduction in circulating levels after splenectomy.<sup>803</sup>

Endogenous pyrogen is produced by human blood monocytes and alveolar macrophages after phagocytosis of bacteria<sup>807</sup> and exposure to other stimuli<sup>545</sup>; new protein synthesis is required and the process is time and temperature dependent.<sup>540</sup>

Lysozyme has been identified histochemically in monocytes as well as neutrophils.<sup>809</sup> Increased levels were observed in the liver, spleen, blood, and kidneys during the period of increased resistance to infection induced by the administration of BCG to mice,<sup>827</sup> and very high levels have been measured in the serum and urine of patients with myelomonocytic leukemia.<sup>550</sup>

Macrophages and blood monocytes of man also have been shown to be a source of colony-stimulating factor<sup>546a</sup>; presumably this material is synthesized by them and secreted.<sup>828</sup>

### Other Secretory Reactions of Monocytes

The monocyte also appears to be essential for the mixed leukocyte culture reaction<sup>829</sup> and lymphocyte transformation.<sup>813</sup> Small numbers of viable, sensitized monocytes (1

per 100 lymphocytes) after exposure to antigen produce an RNA species that is transmitted to lymphocytes by direct cell contact, thus mediating lymphocyte transformation. The process is demonstrable only when lymphocytes and monocytes from the same individual are used.<sup>813</sup>

After the ingestion of silica particles, macrophages release a substance that stimulates collagen formation by fibroblasts.<sup>831</sup> Although not yet characterized, this factor is produced in macrophages damaged by the ingestion of certain toxic particles; it is not present in normal macrophages.<sup>803</sup>

In addition to the above, it is clear that macrophages contribute to the antibody response by trapping antigen in lymph nodes and other sites, presumably by pinocytosis, phagocytosis, and/or retention of antigen on membrane surfaces. By some means the antigen may be processed into more antigenic forms and these apparently can be stored for long periods or transmitted to immunocompetent cells to stimulate antibody production. The details of these processes are as yet poorly understood. They have been reviewed in several articles and books.<sup>808,823,825,841,848,856</sup>

The role of monocytes and macrophages in cellular immunity also is of great importance to body defenses.<sup>835</sup> It is now known that the injection of antigen into previously sensitized animals activates sensitive cells (lymphocytes) to release a variety of substances that recruit nonsensitive monocytes and macrophages to the area.<sup>821,858</sup> A common example of this process of delayed hypersensitivity is provided by the tuberculin skin test.

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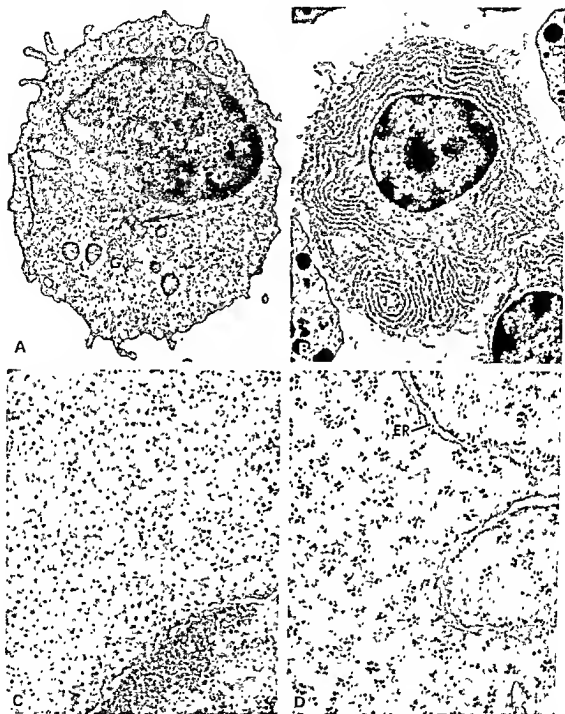


Fig 7-1 A, Lymphocyte obtained from normal peripheral blood. Arrow indicates centriole. The cytoplasm shows mostly single ribosomes and few profiles of rough endoplasmic reticulum (ER). G = Golgi zone (Magnification X13 500). B Plasma cell shows cytoplasm filled with rough endoplasmic reticulum and polyribosomes shown at higher magnification in D (Magnification X6000). C, Detail of the cytoplasm of a resting lymphocyte, possessing mostly single ribosomes should be compared with D which shows the cytoplasm of a stimulated lymphocyte (Magnification X46,000). D, Detail of the cytoplasm of a stimulated lymphocyte or lymphocytoid plasma cell shows polyribosomes and profiles of rough ER. This is also typical for a cell that has responded to antigen or any mitogenic agent (Magnification X46,000) (Courtesy of Dr Dorothea Zucker-Franklin).

have been seen *in vivo* under conditions of antigenic stimulation.<sup>1,13</sup> Transformation is accompanied by a number of well-defined biochemical changes (page 341). Morphologically, such cells more closely resemble large lymphocytes. They range in diameter from 10 to 20  $\mu\text{m}$  and may display bizarre shapes. The nucleus is relatively large and leptochromatic, and contains one or more large nucleoli. The cytoplasm usually is abundant and intensely basophilic. The EM appearance of stimulated lymphocytes, and particularly that of their nuclear components, depends on the mitotic phase of the cell cycle.<sup>21,31</sup> The endoplasmic reticulum is not strikingly developed in PHA-stimulated cells, but it is in those triggered by other agents such as pokeweed mitogen (PWM).<sup>9</sup> The cytoplasm also contains numerous ribosomes and polyribosomes, an expanded Golgi apparatus, and an increased number of mitochondria as well as lysosomes, lipid inclusions, and vacuoles. Mitotic forms are frequent.

### Plasma Cells

Plasma cells probably are progeny of lymphocytes<sup>8</sup> although some contend that they constitute a separate and independent cell line.<sup>17</sup> Morphologically, they are easily differentiated from other cell types (Plate VII). The cells are spherical or ellipsoidal and range from 5 to 30  $\mu\text{m}$  in size. The cytoplasm is abundant and is always basophilic, usually deep blue; it may have a granular character. Plasma cells have a well-defined perinuclear clear zone which contains the Golgi apparatus. In supravital prepared films, the cytoplasm of plasma cells is deep yellowish gray. The nucleus is small in relation to the cell size; it is round or oval, eccentrically placed, and contains dense masses of chromatin, often arranged in a wheel-spoke fashion ("Radkern").

With the *electron microscope* (Fig. 7-1B) the surface membrane and the nucleus appear similar to those of the lymphocyte. The cytoplasm of the plasma cell is characterized by a well-developed rough endoplasmic reticu-

lum which fills most of the cytoplasmic space, except in the area of the perinuclear clear zone which contains the Golgi apparatus. The endoplasmic reticulum consists of parallel lamellae arranged in various patterns, usually in parallel convolutions. The inner surfaces of the lamellae are quite smooth and form the walls of spaces (cisternae) filled with amorphous products of varying density. The outer aspects of the lamellae are rough because of attached ribosomes. A few mitochondria may be seen.

### Intermediate Forms

The above-described EM appearance of lymphocytes and plasma cells is the one most typical of the respective cells. Often, however, *intermediate forms* are found.<sup>31</sup> Thus some cells may resemble small lymphocytes morphologically, but may contain an unusually well-developed rough endoplasmic reticulum. Such "intermediate cells," sometimes referred to as *lymphocytoid plasma cells* or *plasmacytoid lymphocytes*, are common in the blood of patients with plasma cell dyscrasias,<sup>30</sup> and of those with immunologic diseases characterized by hypergammaglobulinemia.<sup>29,30</sup> Similar cells have been encountered in the blood of patients suffering from viral infections ("*Türk cells*");<sup>3</sup> including infectious mononucleosis, as well as in the blood of apparently healthy individuals.<sup>31</sup> Alternatively, immature plasma cells may have an appearance more akin to that of PHA-transformed cells (Fig. 7-2); the nucleus is large and leptochromatic and the cytoplasm contains a rather simple endoplasmic reticulum, but many ribosomes and polyribosomes are present. Thus it is often difficult to draw sharp cytologic dividing lines.

## Histology of Lymphatic Tissues<sup>15</sup>

### Thymus

The thymus is a pinkish gray, lobed mass of tissue lying in the anterior, superior mediastinum. In relation to total body weight, it attains its greatest size during fetal develop-



Fig 7-2 Phytohemagglutinin-stimulated lymphocytes showing characteristic 'blast' cell transformation. The nucleoli are particularly prominent. The cytoplasm develops polysomes, rough ER, and some inclusions of unknown identity (Magnification X3600) (Courtesy of Dr. Dorothea Zucker-Franklin)

ment and the first two years of life. At birth it weighs about 10 to 15 g and it continues to grow (though relatively more slowly than the rest of the body), reaching a maximum of 30 to 40 g at puberty. From then onward it

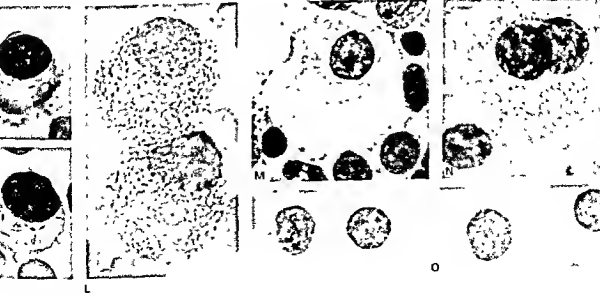
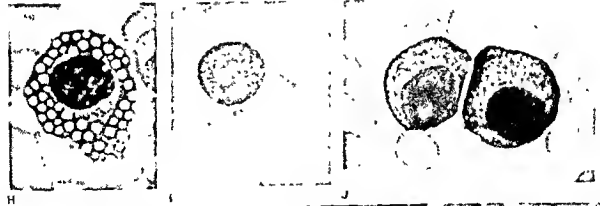
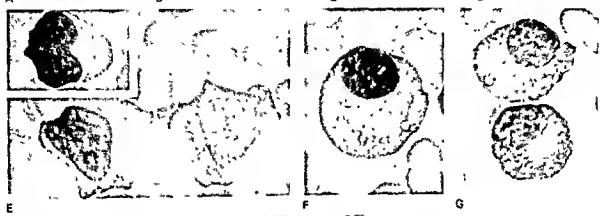
involutesc slowly but remains substantial, even in old age (10 to 15 g), and continues to be very active in lymphopoiesis (see page 301).

The thymic lobes are surrounded by a capsule of connective tissue that sends septa

PLATE VII

- Lymphocytes and plasma cells (Wright's stain, X1500)*
- A. Large and small lymphocytes from the blood of normal subjects
  - B. Lymphocytes resembling plasma cells ("plasmoid") in the blood of a patient with viral pneumonia
  - C. Somewhat atypical lymphocyte and plasmoid lymphocyte in blood
  - D. Lymphocytes from the blood of a patient with viral infection. Azurophilic granules are clearly seen in one of the cells
  - E. Lymphocytes in the blood of a patient with infectious mononucleosis (see also Plate XVII)
  - F. Plasma cell
  - G. Plasmacytes with vacuoles from the bone marrow of a patient with infection and arthritis
  - H to N. Cells in the blood and bone marrow of patients with multiple myeloma
  - H. Plasmacyte with globular bodies ("grape cell"). Bone marrow. Carcinoma of the prostate and IgG myeloma
  - I. Plasma cell with crystalline inclusions. Bone marrow. IgA myeloma
  - J. "Flaming" plasma cells in the blood of a patient with IgA myeloma
  - K. Red-staining crystalline bodies in plasmacytes in IgA myeloma. Bone marrow.
  - L. Plasma cells with heavily vacuolated cytoplasm. IgG myeloma. Bone marrow.
  - M. Plasmacyte showing reticular cytoplasmic structure ("thesaurocyte"). IgA myeloma. Bone marrow
  - N. Plasma cell with two nuclei and reticular cytoplasm. IgG myeloma. Bone marrow.
  - O. Cells from the bone marrow of a patient with macroglobulinemia

# PLATE VII



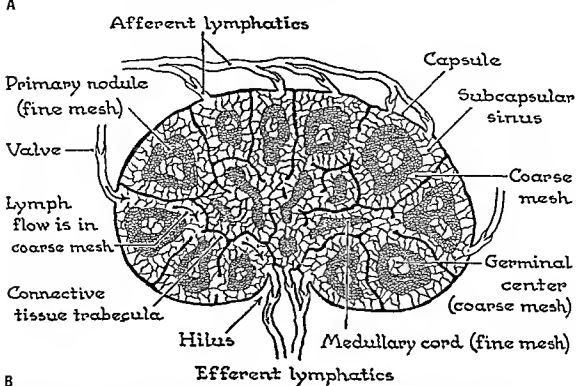


Fig 7-4 Lymph node *A*, Diagram showing the framework of the node as it would appear if the lymphocytes were removed from it *B*, Cross section of a normal lymph node showing lymphatic follicles (LF), germinal centers (GC) medullary cords (MC), and paracortical (PC) lymphoid areas (*A*, from Ham,<sup>15</sup> courtesy of the author and J. B Lippincott Company *B* courtesy of Dr J Hoogstraten, Department of Pediatrics, University of Manitoba)

On section each node can be seen to contain a readily distinguishable cortex and medulla (Fig. 7-4A). The entire structure is surrounded by a connective tissue capsule which sends septa or trabeculae into the substance of the node. The trabeculae provide support and carry blood vessels. The spaces between the trabeculae contain a mesh of *reticular fibers* (Fig. 7-4A). These areas of fine mesh are the sites of "primary follicles" in the cortex and of "medullary cords" in the medulla (Fig. 7-4B). Afferent lymphatic vessels penetrate the capsule to drain lymph into the subcapsular sinus, the latter being a zone of coarse mesh interposed between the capsule and the lymphatic tissue through which fluid percolates easily. The afferent lymphatic vessels start in the tissues as capillaries with blind endings and transport lymph fluid to regional lymph nodes where it is filtered before leaving via the efferent lymphatics. The efferent lymphatics exit on the hilar side to empty into the thoracic or the right lymphatic ducts, which in turn drain into the venous circulation. The centripetal flow of lymph is assured by one-way valves, in both afferent and efferent vessels.

Arteries enter and veins leave lymph nodes at the hilus. The circulation of blood through lymph nodes will be discussed in a subsequent section (page 303).

The cortex contains globular masses of lymphocytes known as *follicles*. In post-natal life (see below) follicles develop a paler looking central area known as a "*germinal center*." Here the cells are larger and have more cytoplasm, and the nuclei therefore appear to be fewer and farther apart. Many mitotic figures may be seen.

In contrast to the cortex, the medulla contains collections of lymphocytes arranged in the shape of cords. They are thought to be extensions of primary follicles into the medulla, and usually contain a large number of plasma cells, which may account for as much as half the cell population of the medullary cords.

The parafollicular areas of lymph nodes also contain small lymphocytes, but they are not arranged in any particular pattern and appear to subserve a different immunologic

function than their follicular fellows (see below).

### Nonencapsulated Lymphatic Nodules

Nonencapsulated lymphatic nodules are dense collections of lymphocytes commonly found in loose connective tissue, most frequently in close proximity to the lining epithelium of the upper respiratory tract, the alimentary tract, and the urinary tract. In some areas, such as the tonsils, the contact between epithelium and lymphoid tissue is most intimate. Because lymphatic nodules are not encapsulated, their periphery is often poorly defined. When they lie in close proximity to each other, as in the Peyer's patches of the lower small bowel, they may appear to be confluent. Nonencapsulated lymphatic nodules may sometimes acquire germinal centers.

### Spleen

A discussion of splenic structure, circulation, and function is found in Chapter 8.

### Bone Marrow

The anatomy of the *bone marrow* is discussed in Chapter 2.

## Development of the Immune System

Current concepts regarding the development and differentiation of the lymphoid system (Fig. 7-5) are derived from critical observations of certain developmental abnormalities of man, and from ontogenic, phylogenetic, and experimental studies of animal systems.

One of the earliest insights into the development of the immune system was provided by the clinical studies of immune deficiency states in man. It was found, for instance, that some individuals developed small lymphocytes normally but lacked plasma cells and antibodies, whereas others had defects of small lymphocytes and cellular immunity but re-

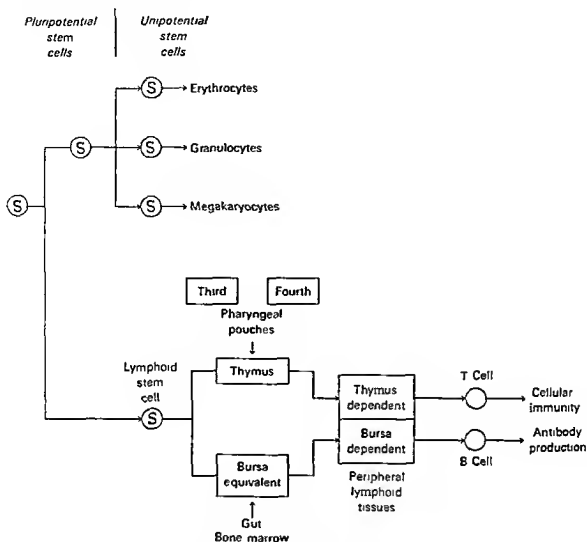


Fig 7-5 The development of the lymphoid system S = stem cell See text for details

tained a normal plasma cell and immunoglobulin system. The first type of defect is best exemplified by Bruton's agammaglobulinemia (see Chapter 44),<sup>36</sup> whereas the selective cellular defect was first discovered in patients with Hodgkin's disease<sup>43</sup> and, later, in patients suffering from the DiGeorge syndrome (Chapter 44). These observations provided the first suggestion of a dichotomy within the immune system, one branch subserving the production of circulating antibodies, the other, cell-mediated immune responses. Still other patients were found to have combined defects, such as those suffering from severe combined immune deficiency

(Chapter 44), and the defects were eventually attributed to absent or malfunctioning stem cells that normally feed both branches of the immune response.

Additional insights came from recognition of the fact that the cellular or humoral immune responses of experimental animals could be ablated selectively.<sup>40,41</sup> Thus it was found that the *bursa of Fabricius*, a hindgut lymphoid organ lying adjacent to the cloaca of birds, was essential for the development of humoral immunity<sup>43</sup> and that its removal, especially when followed by nearly lethal irradiation in newly hatched chicks, led to an absence of plasma cells, immunoglobulins,

and germinal centers in the spleen.<sup>38,40</sup> In addition, these birds could not be induced to produce antibodies, although they maintained normal levels of circulating lymphocytes and rejected foreign skin grafts with vigor. Bursectomized-irradiated chickens subsequently injected with bursal lymphocytes developed fairly normal numbers of plasma cells and germinal centers and produced immunoglobulins, although antibody production remained impaired.<sup>39</sup> Partial restoration could also be achieved by implanting bursal tissue within millipore chambers, suggesting that the bursa may also elaborate a humoral substance that acts on peripheral lymphoid cells.<sup>43</sup>

On the other hand, newly hatched birds irradiated and *thymectomized*, instead of being bursectomized, were found to have low circulating lymphocyte counts and their splenic white pulp was found to be depleted of small lymphocytes. Functionally, cellular immune responses such as delayed hypersensitivity reactions, homograft rejection, and graft-versus-host disease (see page 325 for definitions of these terms) were impaired.<sup>40</sup> These animals did, however, develop plasma cells and germinal centers and produced antibodies, albeit at significantly reduced titers.<sup>38</sup> Similar findings were noted in neonatally thymectomized rodents such as mice, rats, hamsters, and rabbits<sup>47</sup>; generally speaking, cell-mediated immune responses were greatly impaired, but only some circulating antibody responses were similarly affected. The latter usually involve thymus dependent antigens such as sheep red cells and bovine serum albumin,<sup>47</sup> ie, antigens that require the co-operation of thymus dependent and bursa dependent cells in antibody production (see page 316). Neonatally thymectomized animals give near-normal responses to thymus independent antigens such as pneumococcal polysaccharides and *Salmonella* flagellar antigen.<sup>47</sup> At least partial restoration of thymic function can be achieved by implanting thymic tissue in cell-tight millipore chambers, thus suggesting a thymic humoral mediator.<sup>46,48,49a</sup>

Although mammals do not have a bursa,

there is good evidence that, in the rabbit at least, gut-associated lymphoid tissue found in Peyer's patches, the appendix, and the sacculus rotundus may serve as bursal equivalents.<sup>41</sup> Others have suggested that the bursal equivalent of mammals may be dispersed throughout the body or that the bone marrow itself may be such a site.<sup>35</sup>

On the basis of these and other observations,<sup>41,45</sup> Good and his coworkers constructed the following scheme of lymphoid development (Fig. 7-5): Multipotential, undifferentiated stem cells from such sources as the area vasculosa of the yolk sac, the fetal bone marrow, or the fetal liver are thought to be capable of developing along several distinct lines depending on the specialized epithelia with which they come in contact. The epithelial components of the thymus and the bursa (or bursal equivalents) are thought to provide a microenvironment suitable for such differentiation. To emphasize the critical developmental role of these gut-associated tissues, the thymus and bursa, as well as the bone marrow, are referred to as "*central*" or "*primary*" lymphoid organs. Lymphoid stem cells coming into contact with thymic epithelium would be induced to differentiate into small lymphocytes capable of mediating cellular responses, whereas lymphoid precursor cells coming under the influence of the bursa or its mammalian equivalent would eventually differentiate into antibody-producing plasma cells.

The central lymphoid tissues serve to populate and maintain well-defined representative areas in "*peripheral*" or "*secondary*" lymphoid tissues such as the lymph nodes and the spleen; the thymus is considered to populate and maintain the paracortical areas of lymph nodes and the white pulp of the spleen, which are known to subserve cellular immune function, including delayed hypersensitivity reactions, homograft rejection, graft-versus-host disease, and helper functions in certain types of antibody responses (page 316). The bursa or its equivalent is thought to be responsible for populating and maintaining the lymphatic follicles of lymph nodes and spleen, as well as the medullary cords of



Table 7-1. Distinguishing Features of T and B Lymphocytes\*

	<i>T</i>	<i>B</i>
<i>Origin</i>	MARROW → THYMUS	MARROW (→ BURSA†)
<i>Ecotaxis</i>		
Lymph nodes	Paracortical (Perifollicular)	Germinal centers
Spleen	Periarteriolar	Medullary cords
Peyer's patches	Perifollicular	Germinal centers
<i>Ethology</i>	Recirculating	Red pulp
<i>Life span</i>	Long	Central follicles
<i>Surface receptors</i>	Antigen receptors— nature unknown	Sedentary
	Mitogen receptors	Short
	Sheep red cell receptor	Antigen receptors— immunoglobulin
		Complement receptors
		Fc receptors
		Mitogen receptors
<i>Surface configuration</i> (Fig. 7-6)	Smooth	Villous
<i>Response to antigen</i>		
	a Antigen recognition	a Antigen recognition
	b Proliferation	b Proliferation
	c Helper and regulatory function in relation to B cells	c Differentiation into antibody- producing cells
	d Effector cells in cellular immunity	d Establish immunologic memory
	e Establish immunologic memory	
	f May be rendered specifically tolerant	e May be rendered specifically tolerant

\*See text for references and details

†In birds

lymph nodes, all of which are concerned with antibody production. In addition to supplying precursor cells, the central lymphoid organs may also provide humoral substances that promote maturation of immunocompetent cells. The crucial link between the central and peripheral areas on the one hand and their respective functions on the other is provided by extirpation experiments, the study of human immune deficiency states, and the immunization of animals by techniques that stimulate either antibody production or cellular immunity, with ensuing characteristic morphologic changes in the appropriate thymus or bursa (equivalent) dependent areas.

### T and B Cells

Differentiated lymphocytes subserving antibody production are now referred to as *B cells* (bursa dependent or bone marrow dependent cells) whereas those involved in

cellular immune functions (see below) are referred to as *T cells* (thymus dependent cells). The structural and functional properties of these cells are summarized in Table 7-1 and will be discussed in subsequent sections of this chapter.

In the laboratory T cells and B cells are most readily identified by the presence or lack of distinguishing *surface markers*.<sup>33a,35b,42,43a</sup> Thus *B cells* are characterized by the presence of membrane-bound immunoglobulin (Ig), which may be identified by immunofluorescence or other techniques.<sup>35b,42</sup> In the peripheral blood, 10 to 20% of lymphocytes have membrane-bound Ig.<sup>42</sup> IgM (see page 311) appears to be the predominant immunoglobulin class (one half to two thirds) and IgG accounts for most of the remainder. IgA, IgD, and IgE are rarely found.<sup>42</sup> Approximately 25 to 50% of lymphocytes found in lymph nodes, tonsils, and the spleen also bear Ig on their surface.<sup>42</sup> In addition, *B cells* are

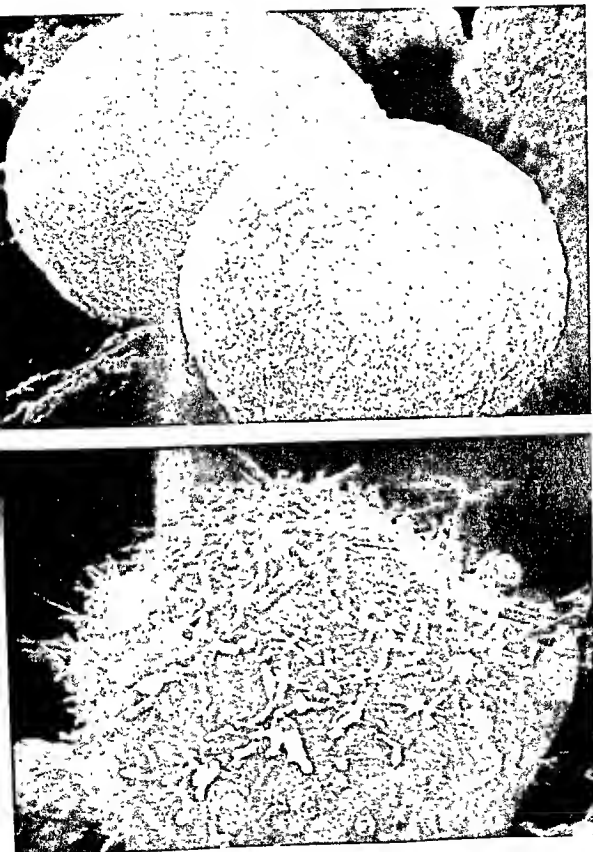


Fig 7-6. Scanning electron micrograph of T cells (*top*) and B cells (*bottom*) (From Pollack et al,<sup>42a</sup> courtesy of the authors and the Journal of Experimental Medicine)

characterized by the presence of receptors for complement (C3) (page 334), but only one half of the peripheral blood cells bearing Ig markers can be so identified.<sup>35b</sup> Another useful distinguishing characteristic is the presence of a receptor for the Fc portion (see page 306) of Ig. This receptor is best demonstrated by the uptake of aggregated gamma globulin on the cell surface.<sup>35b,42</sup>

T cells lack readily identifiable surface immunoglobulin, Fc receptors, or complement receptors. They are most readily identified by their ability to form rosettes with sheep red blood cells, presumably because of the presence of a specific (non-antibody) receptor for such cells.<sup>35a,35b,42</sup> Approximately 69 to 82% of peripheral blood lymphocytes form such rosettes.<sup>35b</sup> B cells do not form sheep red cell rosettes.<sup>35a</sup> Unfortunately a specific T cell marker, such as the  $\theta$  (theta) antigen of mice,<sup>43b</sup> has not yet been identified in man, although several laboratories have succeeded in producing anti-T cell sera of considerable specificity.<sup>35b</sup>

B and T lymphocytes may also be distinguished on the basis of their appearance under the scanning electron microscope (Figure 7-6). T cells show a generally smooth surface, whereas B cells are coated with microvilli.<sup>48a</sup>

## Lymphokines

### Stem Cells

Since a significant number of lymphocytes die or are lost each day, while the total number of cells remains relatively constant, an efficient way of replacing cell loss must exist. The source of cell replacement is the stem cell compartment, the general characteristics of which are described in Chapter 2.

Inferential evidence suggests that hematopoietic stem cell compartments exist at various levels of differentiation (Chapter 2). Thus it appears that lymphocytes ultimately descend from primitive pluripotential stem cells arising in the area vasculosa of the yolk sac.<sup>99</sup> Such cells seem capable of differentiating further into stem cells with more restricted capabilities, such as those that feed into the lymphoid system and those that give rise to

other hematopoietic cells<sup>91</sup> (Fig. 7-5). Finally, lymphocytes themselves appear to form a highly differentiated stem cell compartment since they are capable of blastic transformation, cell division, and eventually reversion to small lymphocytes (page 341).

It is probable that under normal circumstances, the most mature unipotential stem cells feed the lymphoid, erythrocytic, and other compartments. If one of these mature stem cell compartments is damaged, or if there is an excessive demand for cells, more and more primitive progenitors are called upon to feed into the more differentiated compartments.

In the adult, pluripotential hematopoietic stem cells capable of lymphoid and non-lymphoid development, as well as more highly differentiated stem cells, occur in the marrow,<sup>75,91,115</sup> and to a lesser extent in the blood.<sup>54,91</sup> They are also found in the fetal bone marrow and liver<sup>78</sup> but not in other lymphoid tissues.<sup>91</sup>

### Lymphocytes

#### Total Mass

The lymphoid tissues in the aggregate constitute a major organ system,<sup>20</sup> but precise measurements of the total number of lymphocytes in the body of man have not been performed because of the distribution of lymphocytes among a large number of anatomical compartments and their dispersion in organs such as the liver, lungs, and skin.

In rats, attempts have been made to quantitate the total lymphoid mass by combined measurements of organ volumes and estimates of lymphocyte numbers in tissue sections.<sup>83</sup> Others have combined differential counting of tissues containing lymphoid and nonlymphoid cell lines with determinations of the total organ DNA content. The latter, because of its fixed value in diploid cells, provides an accurate estimate of total cell number.<sup>86</sup> With the latter technique it has been estimated that the lymph nodes, thymus, and spleen of rats weighing 200 g contain about  $4 \times 10^9$  lymphoid cells,<sup>86</sup> and that the total lymphoid mass of young rats is of the order

of 0.5 to 1.0% of body weight.<sup>51</sup> These figures probably represent underestimates.<sup>123</sup>

In the calf, estimates of total cell numbers based on prolonged thoracic duct drainage indicate that the total population of lymphocytes exceeds  $10^{12}$  cells.<sup>20</sup> Cannulation of the human thoracic duct yields up to  $3.5 \times 10^{11}$  lymphocytes during the first 60 days of drainage, and about  $0.8 \times 10^9$  daily thereafter.<sup>109</sup> Since these lymphocytes represent only one of the major lymphatic compartments (page 303), it is likely that the total lymphocyte population of adult man also exceeds  $10^{12}$  cells, or about 1 kg of tissue.

### *Techniques for Studying Lymphocyte Kinetics*

**MITOTIC NUMBERS.** Kindred<sup>98</sup> studied the mitotic index of lymphoid tissues. Others have used the rate of metaphase accumulation after mitotic arrest to estimate mitotic activity.<sup>67,87</sup> Knowing the mitotic index ( $m$ ) (expressed as percent of cells in mitosis), the size of the precursor pool ( $N$ ), and the mitotic time in hours ( $t$ ), it is possible to calculate lymphocyte production ( $P$ ) for any organ according to the formula  $P = N \frac{m}{t}$ . The mitotic index may also be used to estimate the "turnover time" of cell populations, i.e., the time it would take to renew all cells within a population, assuming, of course, that all cells within a given compartment have the same proliferative potential. Thus, if the mitotic index of the superficial thymic cortex of rats four hours after administration of colchicine is 4.106%, the turnover time for the entire compartment would be 4.05 days.<sup>87</sup> Values calculated in the same species for other compartments include the following: deep thymic cortex 5.50 days, thymic medulla 13.08 days, lymph node germinal centers 2.40 days, spleen germinal centers 1.79 days, and medullary cord 8.66 days. Turnover times for the outer zone of lymphoid follicles and the paracortical areas could not be calculated because of negligible mitotic activity in these areas. The mitotic indices of peripheral blood and thoracic duct lymphocytes are also low<sup>61</sup>; thus few of these cells are dividing.

**ISOTOPIC DNA LABELING.** Isotopic DNA labeling techniques depend on the incorporation of labeled precursors into the DNA of DNA synthesizing cells. Early studies employed radioactively labeled phosphates ( $^{32}\text{PO}_4$ ),<sup>123</sup> but more recent studies have utilized isotopically labeled thymidine ( $^3\text{H}$  or  $^{14}\text{C}$ ) predominantly.<sup>68,507</sup> This precursor is particularly useful, since its incorporation into DNA may be assessed by radioautographic techniques as well as by the determination of DNA specific activity. It has been used in several different types of studies: (1) If labeled thymidine is given as a single intravenous injection, its relatively rapid clearance from the circulation results in the flash labeling of a cohort of cells synthesizing DNA at that particular time. (2) If the precursor is continuously infused over a period of days or weeks, it will be incorporated into a progressively larger population of cells, as more and more of them pass through the DNA synthetic phase. The rate of increase and the time required to label all cells reflect the rate of renewal in the system. This use of  $^3\text{HTdR}$  is illustrated in Figure 7-7 which shows the rate of appearance of labeled lymphocytes in various tissues during a prolonged period of  $^3\text{HTdR}$  infusion<sup>68</sup>: The small lymphocytes of bone marrow showed the fastest rate of turnover; essentially 100% of these cells were labeled after 4 days, and their half-time renewal rate was 24 hours. The thymus was second with a half-time renewal of 36 hours, followed by spleen, mesenteric lymph nodes, and, finally, thoracic duct lymph. The blood is the only tissue that shows a sharp break in the slope of the labeling curve at four to five days. The two parts of the curve were thought to reflect two populations of cells, each with different life spans (below). (3) Once a cell has been labeled, the dilution of the label by newly synthesized DNA can be used as an index of cell division. The dilution of the label may be determined by radioautography for single cells, or by determining DNA specific activity for populations of cells. However, reutilization of the label complicates interpretation.<sup>109</sup> (4) Isotopically labeled cells may be used to trace their migration patterns. Cells may be labeled

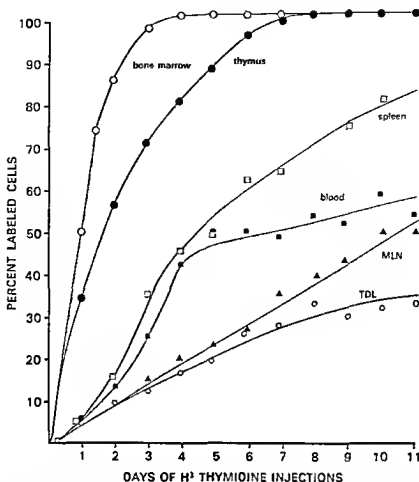


Fig 7-7 The rate of appearance of labeled lymphocytes in various lymphoid compartments in rats during the continuous infusion of tritiated thymidine ( $^3\text{HTdR}$ ). MLN refers to mesenteric lymph nodes, TDL, to thoracic duct lymphocytes (From Everett and Tyler<sup>48</sup> courtesy of the authors and International Review of Cytology)

in vitro and re injected, or specific compartments such as thymus or bone marrow may be labeled by careful local injection of precursors. (5) Precursors such as  $^3\text{HTdR}$  are also useful for the assessment of lymphocyte proliferation under in vitro conditions (page 341).

All these techniques have been used to define the kinetics of lymphopoiesis in various lymphocyte compartments such as the bone marrow, the thymus, the lymph nodes, and the peripheral circulation.

### The Life Span of Lymphocytes

When applied to cells capable of self replication (such as lymphocytes), the term "life

span" usually refers to the intermitotic interval. Sometimes the term is applied to the time between cell division and death, of necessity related events, since mitosis must be followed by the eventual elimination of one of the resulting pair if the total number of cells is to remain constant.

Based on the mistaken belief that thoracic duct lymphocytes are newly formed and on the discovery that the number entering the blood daily from that source is sufficient to replace the peripheral blood lymphocytes several times each day, it was believed for many years that the life span of lymphocytes did not exceed three to four days.<sup>20</sup> In 1954 Ottesen<sup>101</sup> deduced the existence of two populations of cells from experiments in which

he administered  $^{32}\text{P}$  to human subjects and observed the subsequent decay of radioactivity in DNA isolated from peripheral blood lymphocytes: 20% had a mean life span of two to three days, and the remainder a mean life span of 200 to 300 days. Others showed that few lymphocytes from the blood or thoracic duct became labeled after single injections of labeled thymidine, in both *man* and laboratory animals, again suggesting that these cells have a long life span (intermitotic interval).<sup>11</sup>

The studies of Ottesen and others were criticized on the grounds that the long persistence of label in some cells might simply indicate reutilization of the label by succeeding generations of cells.<sup>11</sup> These criticisms did not apply to studies in which label was infused continuously and the number of unlabeled cells was determined. In one such study it was found that after 220 days of continuous infusion of  $^3\text{HTdR}$  into rats, 10% of circulating lymphocytes were still unlabeled and that in one animal 5% remained unlabeled after 270 days.<sup>106</sup> From estimates of the rate at which labeled cells accumulated after repeated injections, it was calculated that in the rat, 90% of the small lymphocytes of the thoracic duct were long-lived, whereas the corresponding figures for blood, mesenteric lymph nodes, and spleen were 66%, 75%, and 25%, respectively. Almost all the small lymphocytes in the thymus and bone marrow were short-lived.

In man the majority of circulating lymphocytes are long-lived. The average life span (intermitotic period) of these cells has been estimated at 4.4 years<sup>56</sup> and for some it may exceed 20 years.<sup>101,102</sup> The life span of short-lived cells is approximately three days.<sup>104,123</sup>

## Lymphopoiesis

### Bone Marrow

The bone marrow is a site of vigorous lymphopoiesis. Radioautographic data from rodents indicate that under steady state conditions virtually all marrow lymphocytes are formed *in situ* and that the entire population

of small lymphocytes in the marrow is renewed in three days or less.<sup>68,103</sup>

From a kinetic standpoint lymphopoiesis in the marrow is similar to that in primary lymphoid organs such as the thymus (see below) and the bursa of birds; the turnover rate is high and it is independent of changes in the peripheral lymphoid system, such as the depletion of recirculating lymphocytes<sup>94,119</sup> and continuous irradiation of the spleen.<sup>72</sup>

The fate and function of most marrow lymphocytes are unknown. (1) Some marrow-derived cells are capable of serving as pluripotent stem cells<sup>91</sup>; however, although it has been suggested that such cells have the morphologic characteristics of lymphocytes,<sup>93</sup> this is far from proven. (2) Others act as lymphoid stem cells, migrating to the thymus, the bursa, or its elusive mammalian counterpart, where they become committed to a particular type of immune function (see page 303). (3) Some migrate to peripheral lymphoid structures and serve as a source of antibody-producing cells ("B cells").<sup>97</sup> (4) The vast majority of marrow lymphocytes, however, have no clearly recognized function and are expended randomly. The high rate of thymidine reutilization within the marrow lymphocyte pool suggests that many, if not most, of these cells disintegrate *in situ*, thereby providing the next generation of lymphocytes with building blocks for DNA synthesis.<sup>62</sup>

### Thymus

The epithelial elements of the thymus develop from the ventral region of the third and fourth pharyngeal pouches. In the human fetus, thymic lymphocytes are first seen at nine weeks of gestation, approximately three weeks before they appear in peripheral lymphoid tissues such as lymph nodes and spleen.<sup>78</sup> The origin of these cells has been a matter of debate. Although some workers in the past have considered thymic lymphocytes to be derived directly from thymic epithelial cells,<sup>52</sup> most investigators favor the view that thymic lymphocytes are the progeny of immigrant cells, most likely derived

from hematopoietic tissues such as the bone marrow or fetal liver (page 295).<sup>47</sup> Such sequestration of circulating lymphoid precursor cells by the thymus has been proved in experiments with thymus grafts, radiation chimeras, and parabionts, using cells carrying chromosome markers or radioactive labels.<sup>47</sup> Lymph node and thoracic duct cells do not normally migrate to the thymus.<sup>71</sup>

Like the marrow, the thymic cortex has a high rate of lymphocyte production. Mitotic indices are about 10 times higher than those of subcutaneous lymph nodes and five times higher than those of mesenteric lymph nodes and Peyer's patches, in both rodents and man.<sup>47,95</sup> The mitotic activity of thymic lymphocytes is highest in the neonatal period and decreases subsequently, especially during the period of involution, but always exceeds that of peripheral lymphoid tissues.<sup>47,95</sup>

About 95% of thymic lymphocytes are replaced every three to four days, whereas the remainder appear to be more long-lived (see below).<sup>47,96</sup> Most thymic lymphocytes probably result from a series of reduction divisions within the cortex, starting with larger, more primitive lymphoid cells.<sup>17</sup> The final products of these sequential divisions, estimated to average between six and eight in the rat, move into the medulla, where some may enter the circulation or lymphatic channels.<sup>107,108</sup> A large number (75%) of the primitive precursor cells in the cortex are replaced continually by immigrant stem cells or lymphoid precursor cells at the rate of about 1% per day; the remainder appear to be of intrinsic origin.<sup>47</sup>

In contrast to the intense proliferative activity of cortical lymphocytes, the lymphoid cells in the thymic medulla exhibit little or no mitotic activity<sup>95</sup>; the small proportion of long-lived lymphocytes found in the thymus may be located in the medulla.<sup>93</sup> Medullary lymphocytes also have other distinguishing features, including a richer supply of mitochondria, a few more sacs of endoplasmic reticulum, and a well-developed nucleolus; greater resistance to radiation and cortisone than cortical lymphocytes; and some degree of immunologic competence.<sup>47</sup>

The exact fate of thymic lymphocytes is

not firmly established. A small number of cells migrate to the "thymus dependent areas" of lymph nodes and spleen (page 295) where they serve as progenitors of cells involved in cell-mediated immune responses and as helper cells in antibody production (T cells) (page 295). Since the total number of thymic lymphocytes remains relatively constant, the remaining cells must either die *in situ*<sup>47</sup> or emigrate from the thymus<sup>108</sup> and die elsewhere.

Little is known of the immunologic factors that regulate thymic lymphopoiesis, including the mechanisms that provide proliferative stimuli and those that limit proliferation and differentiation. It is clear, however, that thymic lymphopoiesis is not subject to external feedback mechanisms such as antigenic stimulation, resection of peripheral lymphoid organs, partial thymectomy, or the presence of thymus grafts, either single or multiple.<sup>47</sup>

Chief among the nonimmunologic factors affecting thymic lymphopoiesis are the adrenal corticosteroids. Adrenalectomy or adrenal failure is associated with an increase in thymic size and mitotic activity,<sup>47,65,66,68</sup> whereas the administration of exogenous corticosteroids or procedures that augment the endogenous production of corticosteroids, such as stress, cause thymic atrophy.<sup>63,111</sup> This occurs because of lymphocytolysis, not increased export of cells.<sup>65</sup> Testosterone and estrogens also may cause acute thymic involution, but this effect is not mediated by the adrenal steroids.<sup>47</sup> Other hormones such as growth hormone and thyroxine cause an increase in thymic size.<sup>47</sup>

### *Peripheral Lymphatic Structures*

The main areas of active lymphopoiesis in normal spleen, lymph nodes, and Peyer's patches are the germinal centers of lymphoid follicles. Follicles are found in the subcortical zone of lymph nodes (Fig. 7-4) and in the white pulp of the spleen (Fig. 8-1). The lymphocytes in the compact cuff surrounding the germinal centers (Fig. 7-4) proliferate at a slower rate and are not primarily derived from the cells in the germinal centers.<sup>70</sup>

The development of germinal centers

depends on antigenic stimulation; they do not appear until late in the course of a true primary immune response,<sup>59</sup> and are most characteristically associated with secondary antigenic stimulation<sup>59,60</sup> (see page 317 for definitions of these terms). Thus germinal centers may originate from pre-sensitized cells and may represent sites of rapid proliferation of these elements.<sup>59</sup>

The rate of lymphopoiesis in germinal centers is high, rivaling that of bone marrow and thymus.<sup>70,89</sup> Some germinal center cells migrate from the heavily proliferating portion to the more loosely structured area of the center<sup>82</sup> and into the surrounding lymphoid tissue.<sup>89</sup> It is likely that at least a fraction of these cells differentiates into antibody-producing cells.<sup>112</sup> However, most of the newly formed cells appear to disintegrate *in situ* and their nuclei and other constituents are frequently ingested by monocytes and macrophages, whose production accompanies the intense lymphoproliferative activity ("tingible body macrophages").<sup>70</sup> The role of germinal centers in antibody production will be discussed in a later section (page 315).

Following stimulation with certain (thymus dependent) types of antigens (see page 316), cells found in the paracortical zones of lymph nodes and in the periarteriolar sheaths of the spleen (both of which are "thymus dependent") are also capable of proliferation.<sup>50,83</sup> These cells are involved in antigen recognition (page 316) and, in certain experimental systems, their proliferation appears to precede germinal center development<sup>83</sup> and the proliferation of antibody-producing cells.<sup>232</sup> (See also section on cell cooperation in immune responses, page 316.) Such cells are also involved in cell-mediated immune responses (page 320). The rate of lymphopoiesis in paracortical and periarteriolar areas is, however, much less than that in the follicles.

### Control of Lymphocyte Production

The major factor controlling the total mass of lymphocytes within the body appears to be the amount of exposure to antigen. Thus, germfree animals have much less lymphoid

tissue as compared to non-germfree controls, even though the immune system is intact.<sup>54a,92</sup>

A variety of factors that influence blood lymphocyte concentration have been described and proposed as humoral regulators of lymphopoiesis.<sup>66a,72a,91a</sup> However, induction of lymphocytosis in the blood does not necessarily indicate an increase in lymphocyte production. It may simply reflect a reduced rate of recirculation with a lengthened intravascular sojourn, such as occurs after injection of pertussis vaccine.<sup>105</sup> Injection of serum from irradiated rats into normal rats was shown to stimulate mitosis in lymphoid tissue<sup>86a</sup> but one cannot be certain that neo-antigens were not induced by irradiation. Thus, proof of a humoral factor specifically governing lymphopoiesis is lacking.<sup>117</sup> The influence of adrenal steroids and other hormones on lymphopoiesis has been discussed (page 302).

### The Circulation of Lymphocytes

Two basic migration patterns of lymphocytes have been identified (Fig. 7-8): one involves the migration of precursor cells from the marrow to the thymus and the bursa and from these to the peripheral lymphoid organs, such as the lymph nodes and the spleen; the other consists of a continuous recirculation of highly differentiated lymphocytes derived primarily from the thymus dependent areas of the lymph nodes and the spleen.

#### Circulation of Precursor Cells

(1) There is a steady migration of rapidly dividing cells from the bone marrow to the thymus,<sup>68,71,108</sup> which may function as a "finishing school" for some immunologically immature cells. For other cells the bursa, at least in birds, may function in a similar capacity.<sup>41,120</sup> (2) Thymus-produced cells in turn migrate to peripheral lymphoid structures where they localize in thymus dependent areas (page 295), and probably contribute to the pool of long-lived lymphocytes subserving T cell function (page 295).<sup>20,47,68</sup> The number of cells migrating from the



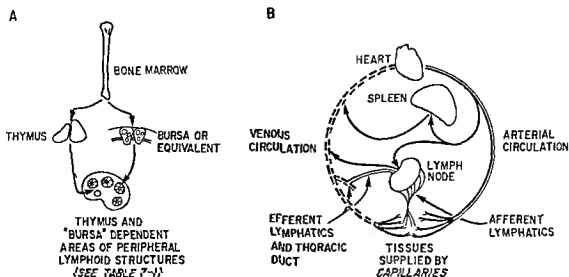


Fig 7-8 Circulation of lymphocytes. *A*, Circulation of precursor cells. *B*, circulation of highly differentiated cells. See text for details.

thymus to peripheral lymphoid structures increases considerably following antigenic stimulation.<sup>47</sup> (3) In birds the migration of cells from the bursa of Fabricius to peripheral lymphoid organs also has been demonstrated.<sup>120</sup>

### Recirculation of Lymphocytes

The long-lived small lymphocytes found in the blood, the lymphatics, and the peripheral lymphoid organs comprise most of the recirculating pool. Functionally they are identified as T cells (page 295) and reside in the thymus dependent areas (page 295) of lymph nodes and spleen. The output of lymphocytes from the thoracic duct of many species, including man, is far in excess of the number present in the blood.<sup>11</sup> The hypothesis that a continuous recirculation of cells between blood and lymph explains this rapid turnover of lymphocytes in the blood has been confirmed. (1) It was shown that the profound fall in the output of lymphocytes which occurs during chronic drainage of lymph from the thoracic duct of rats could be prevented if the lymphocytes flowing from the thoracic duct fistula were continuously reinfused into the blood.<sup>11</sup> Furthermore, it was shown that profound lymphopenia and a sharp reduction in the output of lympho-

cytes from the thoracic duct occurred when lymphocytes were killed by irradiating blood as it passed through an extracorporeal circuit.<sup>63</sup> (2) More definitively, when labeled thoracic duct lymphocytes were injected intravenously, they were found in the thoracic duct and the intestinal lymph and there was no decrease in grain count.<sup>11,53</sup> (3) In parabiotic rats labeled lymphocytes of one member of a pair can be recovered from the thoracic duct of the other<sup>110</sup>; these lymphocytes could only have come from the blood of the other rat. (4) The experiments of Hall and Morris clearly show that the great majority of small lymphocytes leaving a node in the efferent lymph are neither formed there nor come from the afferent lymph.<sup>81</sup> Thus they must have passed into the node from the blood.

Whether all lymphocytes in thymus dependent areas are circulating continuously and equally is not known; some experiments suggest the existence of two compartments of cells<sup>80</sup>; one that is readily mobilized and the other consisting of cells that recirculate slowly, or that are only withdrawn under conditions of special stress such as chronic lymphocyte drainage.

The passage of lymphocytes from blood to lymph occurs within lymph nodes and the spleen.<sup>80,81</sup> When labeled cells are injected

into the blood, they accumulate in high concentration in the deeper part of the cortex of lymph nodes, in Peyer's patches, and in the white pulp of the spleen around the central arteriole.<sup>60</sup> The adult thymus is completely excluded from this type of recirculation. Labeled cells then enter lymphatic channels and cross postcapillary venules by passing between the tall endothelial cells.<sup>109a</sup> The nature of the special affinity that small lymphocytes and postcapillary venules have for each other is not known. It has been suggested that sugar groupings on the surface of lymphocytes may be involved in accurate homing.<sup>77</sup>

## Lymphocyte Function

The chief function of lymphocytes is the generation of immunity, a complex phenomenon culminating in the synthesis of specific immunoglobulins (antibodies) and the establishment of cellular immunity. These processes are of sufficient importance to clinical hematology to warrant an outline of their main characteristics.

### Antibody Formation

#### Immunoglobulin Structure—

##### General Features<sup>135,139,160,161,180</sup>

The antibody activity of serum and secretions is associated with a heterogeneous

group of proteins collectively known as immunoglobulins. Since the structural and functional diversity of normal antibodies makes an analysis of their physicochemical properties difficult, most of our knowledge concerning immunoglobulin structure is derived from studies of homogeneous populations of "abnormal" immunoglobulins such as myeloma proteins and monoclonal macroglobulins.<sup>169</sup>

Despite their tremendous heterogeneity, all antibodies share certain structural similarities. All consist of a basic subunit composed of four polypeptide chains held together by disulfide bonds (Fig. 7-9). Two have a molecular weight of 53,000 to 75,000, depending on the immunoglobulin class, and are known as heavy (H) chains; the other two have a molecular weight of 22,500 and are known as light (L) chains. On the basis of their general properties (Table 7-2), and the physicochemical and immunochemical features of their constituent H chains, immunoglobulins may be subdivided into five major classes: IgG, IgM, IgA, IgD, and IgE (Table 7-2). The respective heavy chains are designated by the Greek letters  $\gamma$  (IgG),  $\mu$  (IgM),  $\alpha$  (IgA),  $\delta$  (IgD), and  $\epsilon$  (IgE). Within a given class of heavy chains, subclasses have been distinguished by their antigenic characteristics. For instance, there are four subclasses of IgG (page 309).

In contrast only two types of L chains have

Table 7-2. Structural Features of Immunoglobulins

	IgG	IgA	IgM	IgD	IgE
Heavy chains	$\gamma$	$\alpha$	$\mu$	$\delta$	$\epsilon$
Subclasses	1 2 3 4	1, 2	1, 2	—	—
Genetic factors	Gm	Am	—	—	—
Light chains	$\kappa$ or $\lambda$	$\kappa$ or $\lambda$	$\kappa$ or $\lambda$	$\kappa$ or $\lambda$	$\kappa$ or $\lambda$
Other chains	—	SP* JC†	JC†	—	—
Molecular weight	155,000	170,000‡	850,000	180,000	200,000
S <sub>20</sub> × §	7	7 (9 11 13)	19	7	8
Electrophoretic mobility	$\gamma(\beta)$	$\beta$	$\gamma-\beta$	$\gamma-\beta$	$\gamma-\beta$
Carbohydrate, %	2.9	7.5	10.7	12.0	10.7

\*Secretory piece

†J chain

‡In secretions MW = 390,000

§Sedimentation rate in Svedberg units

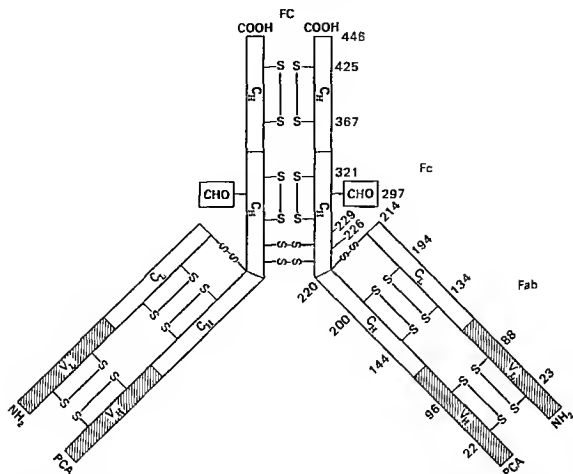


Fig. 7-9 Gammaglobulin structure (IgG1) after data of Edelman.<sup>13a</sup> Variable regions of both light and heavy chains are shaded. Constant regions are not. Numbering of amino acid residues proceeds from N terminal and of each chain.

been identified,  $\kappa$  and  $\lambda$ . These chains are shared by all classes of immunoglobulins, although the relative amounts of each vary from class to class. However, any given immunoglobulin molecule has either  $\kappa$  or  $\lambda$ , never both.

Each heavy chain is joined to its adjacent light chain by noncovalent forces and a single disulfide bond. The two light-heavy chain pairs of each immunoglobulin molecule are also linked by noncovalent interactions and by neighboring disulfide bonds (usually two) between the heavy chains. Treatment of immunoglobulins with reducing agents breaks the disulfide bonds.

When a 7S gammaglobulin molecule is

treated with papain in the presence of cysteine, three 3.5S fragments result (Fig. 7-10b)<sup>170</sup>; two of these are identical and consist of a light chain and the amino-terminal end of the heavy chain (the Fd fragment of heavy chain); these are referred to as Fab (antigen binding) fragments. The third piece, known as the Fc (crystallizable) fragment, contains the carboxy-terminal half of both H chains, most of the carbohydrate, and the antigens determining class specificity (eg,  $\gamma$ ,  $\alpha$ ,  $\mu$ , etc). The Fc piece mediates fixation of the immunoglobulin molecule to cells (eg, skin, mast cells, lymphocytes, macrophages), placental transfer, and complement fixation. When 7S is treated with pepsin rather than

papain,<sup>139</sup> one large piece with a molecular weight of 100,000 and many small peptide fragments are obtained. The small fragments are derived from a more complete degradation of the Fc portion, whereas the large fragment is found to consist of two Fab fragments joined by disulfide bonds [F(ab)<sub>2</sub>].

*L chains* are composed of 214 amino acid residues.<sup>171</sup> The chains can be viewed as having two looped regions of equal length (Figs. 7-9 and 7-11). The first of these regions

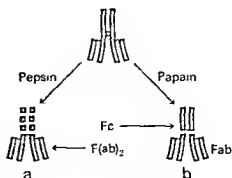
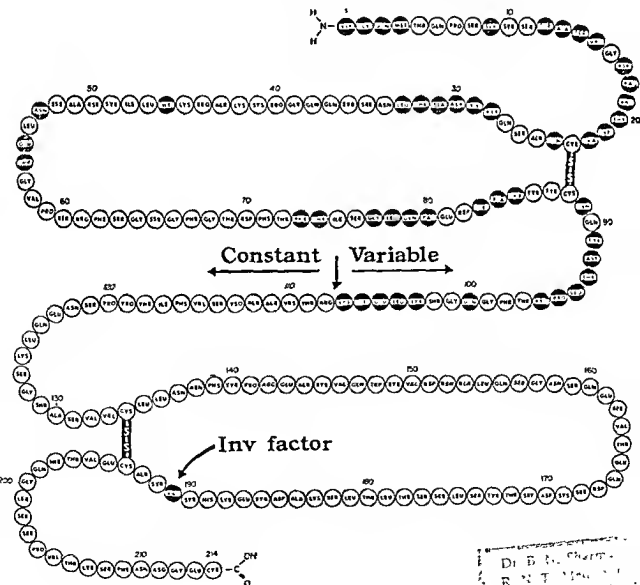


Fig. 7-10. Immunoglobulin fragments produced by pepsin (a) and papain (b)

Fig. 7-11. Amino acid sequence of human kappa Bence Jones protein. The black circles mark variable loci, where different amino acids have been found in other human kappa Bence Jones proteins. Note loops formed by disulfide bridges (From Putnam et al,<sup>171</sup> courtesy of the authors and Cold Spring Harbor Symposia)



Dr. B. L. Chain  
R. N. T. Chain  
[Signature]

(starting at the amino-terminal end of the chain and including amino acid residues 1 to 107) is referred to as the variable ( $V_L$ ) region, whereas the remainder is referred to as the constant ( $C_L$ ) region.<sup>171</sup> (The subscript L denotes the light chain origin of the region.) The variable half of the chain is so named because its amino acid sequence varies considerably from one immunoglobulin to another, whereas the constant region shows much less variability. Similar regions are found in the *heavy chains*. The chains belonging to the IgG1 class of immunoglobulins have been studied most extensively.<sup>133,136,172</sup> They are composed of 446 amino acid residues and can be viewed as consisting of four separate regions, each containing a loop with an internal disulfide bridge. Comparable to light chains, the first (amino-terminal) region, consisting of about 121 amino acid residues, is the variable portion of the heavy chain ( $V_H$ ), whereas the remaining regions appear to be similar repetitive units and are designated  $C_{H1}$ ,  $C_{H2}$ , and  $C_{H3}$ , beginning with the region next to the variable one. Thus the Fc fragment consists of the  $C_{H2}$  and  $C_{H3}$  homologous regions, whereas the Fab fragment contains the  $V_H$  and  $C_{H1}$  regions of the heavy chain and both regions ( $V_L$  and  $C_L$ ) of the light chain (Fig. 7-10).

The antigen binding sites reside in the amino-terminal half of the antibody molecule, and, more specifically, in its  $V_L$  and  $V_H$  regions.<sup>135,142,161</sup> Both the heavy and the light polypeptide chains are required for antibody activity. Amino acid sequence data have shown that the variable regions of L and H chains contain smaller regions of "hypervariability"<sup>191</sup> that ultimately form the antigen binding site and are responsible for the generation of antibody specificity and diversity.<sup>135,142</sup> For  $V_L$  the hypervariable regions include amino acid positions 24 to 34, 50 to 56, and 89 to 97; for  $V_H$ , positions 31 to 37, 86 to 91, and 101 to 109.<sup>131</sup> It is obvious that even within  $V_H$  and  $V_L$ , the areas of hypervariability are small in comparison to the length of the entire variable regions. Indeed, their name to the contrary,  $V_H$  and  $V_L$

also contain areas of constancy that are thought to maintain the basic tertiary structure of the chain and thereby allow the hypervariable regions to function in antigen binding.<sup>191</sup>

Within the constant region of light chains ( $C_L$ ), certain amino acid substitutions give rise to distinct allotypic markers. When position 191 of  $\kappa$  light chain is occupied by leucine, the InV(1) and InV(2) allotypes result; the valyl residue at this position gives rise to the InV(3) allotype.<sup>181</sup> The antigenicity of these allotypes is expressed only in the intact molecule: cleavage of the  $\kappa$ -chain into its  $V_L$  and  $C_L$  regions results in loss of InV antigenicity.<sup>180</sup>

Structural variations in the amino acid sequence of light ( $\lambda$ ) chains have been noted at two positions, 191 and 154. At position 191 (corresponding to the InV locus of kappa chains), a lysyl residue is associated with the immunochemical classification of Oz (+), arginyl with Oz (-).<sup>137</sup> Lambda chains containing glycine at position 154 have been designated Kern (+) and those containing a serine residue have been designated Kern (-).<sup>143</sup> Neither the Oz nor the Kern antigens are allelic genetic markers, however.<sup>143</sup>

In addition to the InV genetic marker localized to the constant region of kappa chains, over 20 genetic markers are associated with the constant region of  $\gamma$  chains. These markers will be discussed later (page 309).

## IgG

IgG is the major immunoglobulin in man and constitutes about three fourths of the total  $\gamma$  globulins (Table 7-3). The serum concentration varies from 800 to 1600 mg/dl in adults, but the intravascular pool accounts for less than half the total body IgG; about 55% is found widely distributed within the extravascular space. The total body content is in excess of 1 g/kg of body weight.<sup>189</sup> IgG molecules have a half-life of about 21 days and are thus the longest lived immunoglobulins.

On the basis of antigenic determinants within heavy chains of IgG, four isotypic

Table 7-3. Functional Properties of Immunoglobulins

	IgG	IgA	IgM	IgD	IgE
Serum concentration (mg/dl)	1200	250	120	3	0.03
% intravascular distribution	45	42	75	75	50
Fractional catabolic rate (% per day)	6.7	25	18	37	89
Synthesis (mg/kg/day)	33	24	6.7	0.4	0.02
Survival ( $t_{1/2}$ , days)	21	6	5	2.8	2.2
External secretions	+	+++	+		
CSF	+	+	0		
Complement fixation	+	0	+		
Transport across placenta	+	0	0	0	0

subclasses of IgG molecules have been identified in normal serum: IgG1 (66%), IgG2 (23%), IgG3 (7%), and IgG4 (4%).<sup>165</sup> These antigenic differences are the result of variations in amino acid sequences of the carboxy-terminal parts of  $\gamma$ -chains. All four types of molecules are found in any given normal serum, but individual molecules contain only a single type of  $\gamma$ -chain. Similar distributions are found among myeloma proteins.<sup>165</sup> Since these antigenic (isotypic) differences among IgG molecules carry functional implications (see below), they are of more than serologic interest.

In addition to the antigenic differences that determine immunoglobulin subclasses, proteins within each IgG subclass possess distinctive allotypic antigens known as Gm factors. (The corresponding antigens on IgA molecules are known as Am factors.<sup>165,180</sup>) These factors are the products of allelic genes that are associated with the separate cistrons controlling each immunoglobulin subclass.<sup>139</sup> Table 7-4 lists the common markers associated with the IgG1, IgG2, IgG3, and IgA2 subclasses. The IgG4 subclass has no recognized Gm markers.

#### Biologic Properties of IgG

Most of the antibodies developing in secondary responses (page 317) to antigen are

IgG. It is the only immunoglobulin selectively transferred across the placenta, thereby giving a measure of protection to the newborn infant.<sup>162,190</sup>

Some biologic properties of IgG proteins, and particularly those mediated by the Fc fragment, are distinctly subclass specific. Thus complement activation through binding of C1q, the first activated component of complement (page 333), is most efficient with IgG1 and IgG3. IgG2 also is active but IgG4 proteins are completely inactive.<sup>150,163</sup> The binding to macrophage and granulocyte Fc receptors is most efficient with IgG1 and IgG3.<sup>125,146,156</sup> The latter reaction is of importance in opsonization (Chapter 8). Anti-immunoglobulin antibodies (rheumatoid factors) react most readily with IgG1, IgG2, and IgG4 proteins, but not with IgG3 proteins.<sup>165</sup> This reaction appears to be due to the presence of a site called Ga which is found on all subclasses except IgG3. IgG3, on the other hand, has shown a great tendency to aggregation,<sup>132</sup> which is probably involved in its affinity for C1q.<sup>163</sup> Aggregation of IgG3 may also lead to clinically significant hyperviscosity states.<sup>132</sup> In addition, IgG3 is selectively retained in the sera of a number of patients with generalized hypogammaglobulinemia.<sup>523</sup>

Some antibody activities are subclass specific. Thus anti-Rh antibodies are usually

**Table 7-4. Immunoglobulin Heavy Chain Allotypes and Relation to Immunoglobulin Classes and Subclasses.**

	Nomenclature		Subclass of Heavy Chain
	New	Original	
Gm markers			
1		a	IgG1
2		x	IgG1
3		b <sup>w</sup> or b <sup>z</sup>	IgG1
4		f	IgG1
5		b and b <sup>z</sup>	IgG3
6		c	IgG3
7		r	IgG1
8		e	
9		p	
10		b <sup>a</sup>	IgG3
11		b <sup>g</sup>	IgG3
12		b <sup>y</sup>	IgG3
13		b <sup>z</sup>	IgG3
14		b <sup>4</sup>	IgG3
15		s	IgG3
16		t	IgG3
17		z	IgG1
18		Rouen 2	IgG1
19		Rouen 3	?
20		San Francisco 2	IgG1
21		g	IgG3
22		y	
23		n	IgG2
		b <sup>0</sup>	IgG3
		b <sup>3</sup>	IgG3
		c <sup>3</sup>	IgG3
		c <sup>5</sup>	IgG3
Am markers			
1		1 or +	IgA2

From Natvig and Kunkel<sup>145</sup> courtesy of the authors and *Advances in Immunology*

IgG1 or IgG3 (Chapter 27), antifactor VIII antibodies (Chapter 38) are often restricted to IgG4, and other antibodies, such as anti-dextran and antilevan, have been found to be IgG2.<sup>165</sup>

## IgA

The IgA class of antibodies can be divided into two separate systems of immunoglobulins.<sup>129,186</sup> One of these provides IgA antibodies for the *circulation* and the *internal*

secretions such as the aqueous humor of the eye, the cerebrospinal fluid, and the synovial, amniotic, pleural, and peritoneal fluids. It is likely that these IgA antibodies are synthesized by plasma cells of organs normally involved in antibody production. The other system of IgA antibodies is found in *external* secretions such as saliva, tears, bile, colostrum, as well as those of the respiratory tract, the gastrointestinal tract, the seminal vesicles, the cervix, and the urinary tract. The IgA of external secretions is, for the most part, not derived from blood, but is produced locally by plasma cells situated in close proximity to the epithelial mucosa.<sup>186</sup> It is the predominant immunoglobulin in external secretions, although smaller amounts of IgM and IgG may also be found.

In the serum the IgA molecule usually is present as a monomer with a molecular weight of 170,000 and a sedimentation coefficient of 7S, but 9S, 11S, and 13S polymers frequently occur. Its serum concentration is in the range of 200 to 300 mg/dl with a  $t_{1/2}$  of six days.<sup>180</sup> On the basis of antigenic differences within the  $\alpha$  (heavy) chain, IgA globulins can be divided into two subclasses: IgA1 (93%) and IgA2 (7%).<sup>139</sup> In the IgA2 subclass the H-L bond may not be of the disulfide variety, since light chains are liberated in acid, frequently in the form of dimers.<sup>139</sup> The last observation suggests that the L chains may be situated close to each other, perhaps on the inside of the molecular crotch.

Secretory IgA is a large molecule with a molecular weight of 390,000 to 400,000 and a sedimentation coefficient of 11S.<sup>185</sup> It generally consists of a dimer of two IgA molecules and an additional nonimmunoglobulin component known as *secretory piece* or *transport (T) piece*.<sup>186</sup> This component has a molecular weight of 58,000 and is structurally and genetically unrelated to immunoglobulins.<sup>186</sup> The secretory piece is made in epithelial cells rather than in plasma cells and appears to link up with IgA molecules during their transport across the mucosa or within secretions.<sup>185</sup> The function of the secretory

piece is not clear. It appears to have no role in IgA transport,<sup>186</sup> but it may stabilize the molecule and may protect it against proteolysis within secretions, especially those of the intestinal tract.<sup>186</sup>

Another polypeptide chain, designated the *J* chain ("joining" chain), has been detected in association with polymeric forms of serum and secretory IgA, as well as with the IgM pentamer (see below).<sup>145, 157, 158</sup> This chain has a molecular weight of 23,000 to 26,000. It is attached to IgA or IgM polymers by disulfide linkages. Only one *J* chain is found per polymer and none is found in association with monomeric immunoglobulins.<sup>158</sup> It is not detectable by antisera while the polymers are in their native state, but it becomes readily accessible if the polymers are dissociated.<sup>152</sup> In contrast to the secretory piece, *J* chains are produced by plasma cells,<sup>167</sup> and they appear to play a key role in the process of polymerization of immunoglobulin.

### Biologic Properties of IgA

Secretory IgA activity against a variety of viruses and bacteria has been reported,<sup>151, 178</sup> but the mechanism of antimicrobial or antiviral action of IgA is unknown. Although IgA is incapable of fixing complement or acting as an opsonin, the secretory IgA molecule together with complement and lysozyme, an enzyme found in all external secretions so far examined, is capable of killing *E. coli*.<sup>129</sup> The effects of IgA lack are described in Chapter 44. It is rather surprising that most deficient individuals appear to be rather healthy and not overly susceptible to upper respiratory infections. IgA antibodies against intrinsic factor have been reported in the gastric secretion of a patient with pernicious anemia.<sup>144</sup>

### IgM

IgM antibodies<sup>159, 172</sup> are proteins with a molecular weight of 850,000 which sediment predominantly at 18S to 19S, but also at 22S, 26S, and 35S. Because of their size, IgM

molecules are referred to as *macroglobulins*. Their rate of synthesis is only one twentieth that of IgG, whereas their fractional catabolic rate is two to three times that of IgG.<sup>189</sup> This accounts for the relatively short survival ( $t_{1/2} = 5$  days) and low serum levels (80 to 90 mg/dl) of IgM.<sup>159</sup>

IgM macromolecules are composed of five identical subunits called IgM monomers, each of which consists of two  $\mu$  (heavy) chains and two light chains. The light chains may be  $\kappa$  or  $\lambda$  and appear to be identical to those of other immunoglobulins. The heavy chains, on the other hand, have some unique structural features<sup>172</sup>; whereas human  $\gamma$ -chains vary in length from 446 to 450 amino acid residues,  $\mu$ -chains contain in excess of 500 residues and have a correspondingly higher molecular weight of about 70,000. In addition  $\mu$ -chains appear to consist of a variable region and four constant regions, in contrast to the three constant regions of  $\gamma$ -chains. Similarly to  $\gamma$ -chains, however, each region (constant and variable) contains a loop of about 60 amino acids with an internal disulfide bridge flanked on either side by about 20 amino acids. Carbohydrates account for 10.7% of the molecule by weight and are distributed over five sites within the constant region: one within the Fd region, one in the hinge region, two within Fc, and one near the COOH terminus.<sup>172</sup> They affect the conformation and other properties of the molecule but do not contribute directly to antibody specificity. None of the glycopeptides of  $\mu$ -chains corresponds to the single glycopeptide of  $\gamma$ -chains. Finally, the variable regions of  $\mu$ - and  $\gamma$ -chains are much more similar (homologous) than are their constant regions. On the basis of these observations it has been suggested that two genes may code for each immunoglobulin heavy chain; one codes for the variable region and possibly for the same antibody specific site of  $\mu$ - and  $\gamma$ -chains, and the other codes for the constant regions of each chain.<sup>172</sup> At least two subclasses of IgM have been identified on the basis of antigenic differences within the  $\mu$ -chain (IgM1, IgM2).<sup>138, 139</sup> The exist-



**Tabla 7-4. Immunoglobulin Heavy Chain Allotypes and Relation to Immunoglobulin Classes and Subclasses.**

	Nomenclature		Subclass of Heavy Chain
	New	Original	
Gm markers			
1		a	IgG1
2		x	IgG1
3		b <sup>+</sup> or b <sup>2</sup>	IgG1
4		f	IgG1
5		b and b <sup>1</sup>	IgG3
6		c	IgG3
7		r	IgG1
8		e	
9		p	
10		b <sup>+</sup>	IgG3
11		b <sup>2</sup>	IgG3
12		b <sup>3</sup>	IgG3
13		b <sup>3</sup>	IgG3
14		b <sup>4</sup>	IgG3
15		s	IgG3
16		t	IgG3
17		z	IgG1
18		Rouen 2	IgG1
19		Rouen 3	?
20		San Francisco 2	IgG1
21		g	IgG3
22		y	
23		n	IgG2
		b <sup>0</sup>	IgG3
		b <sup>1</sup>	IgG3
		c <sup>1</sup>	IgG3
		c <sup>2</sup>	IgG3
Am markers			
1		1 or +	IgA2

From Natvig and Kunkel,<sup>145</sup> courtesy of the authors and *Advances in Immunology*

IgG1 or IgG3 (Chapter 27); antifactor VIII antibodies (Chapter 38) are often restricted to IgG4, and other antibodies, such as anti-dextran and antilevan, have been found to be IgG2.<sup>165</sup>

## IgA

The IgA class of antibodies can be divided into two separate systems of immunoglobulins.<sup>129,186</sup> One of these provides IgA antibodies for the *circulation* and the *internal*

secretions such as the aqueous humor of the eye, the cerebrospinal fluid, and the synovial, amniotic, pleural, and peritoneal fluids. It is likely that these IgA antibodies are synthesized by plasma cells of organs normally involved in antibody production. The other system of IgA antibodies is found in *external* secretions such as saliva, tears, bile, colostrum, as well as those of the respiratory tract, the gastrointestinal tract, the seminal vesicles, the cervix, and the urinary tract. The IgA of external secretions is, for the most part, not derived from blood, but is produced locally by plasma cells situated in close proximity to the epithelial mucosa.<sup>186</sup> It is the predominant immunoglobulin in external secretions, although smaller amounts of IgM and IgG may also be found.

In the serum the IgA molecule usually is present as a monomer with a molecular weight of 170,000 and a sedimentation coefficient of 7S, but 9S, 11S, and 13S polymers frequently occur. Its serum concentration is in the range of 200 to 300 mg/dl with a  $t_{1/2}$  of six days.<sup>180</sup> On the basis of antigenic differences within the  $\alpha$  (heavy) chain, IgA globulins can be divided into two subclasses: IgA1 (93%) and IgA2 (7%).<sup>139</sup> In the IgA2 subclass the H-L bond may not be of the disulfide variety, since light chains are liberated in acid, frequently in the form of dimers.<sup>139</sup> The last observation suggests that the L chains may be situated close to each other, perhaps on the inside of the molecular crotch.

Secretory IgA is a large molecule with a molecular weight of 390,000 to 400,000 and a sedimentation coefficient of 11S.<sup>185</sup> It generally consists of a dimer of two IgA molecules and an additional nonimmunoglobulin component known as *secretory piece* or *transport (T) piece*.<sup>186</sup> This component has a molecular weight of 58,000 and is structurally and genetically unrelated to immunoglobulins.<sup>186</sup> The secretory piece is made in epithelial cells rather than in plasma cells and appears to link up with IgA molecules during their transport across the mucosa or within secretions.<sup>188</sup> The function of the secretory

piece is not clear. It appears to have no role in IgA transport,<sup>186</sup> but it may stabilize the molecule and may protect it against proteolysis within secretions, especially those of the intestinal tract.<sup>186</sup>

Another polypeptide chain, designated the *J* chain ("joining" chain), has been detected in association with polymeric forms of serum and secretory IgA, as well as with the IgM pentamer (see below).<sup>145,157,158</sup> This chain has a molecular weight of 23,000 to 26,000. It is attached to IgA or IgM polymers by disulfide linkages. Only one *J* chain is found per polymer and none is found in association with monomeric immunoglobulins.<sup>158</sup> It is not detectable by antisera while the polymers are in their native state, but it becomes readily accessible if the polymers are dissociated.<sup>152</sup> In contrast to the secretory piece, *J* chains are produced by plasma cells,<sup>167</sup> and they appear to play a key role in the process of polymerization of immunoglobulin.

### *Biologic Properties of IgA*

Secretory IgA activity against a variety of viruses and bacteria has been reported,<sup>151,178</sup> but the mechanism of antimicrobial or antiviral action of IgA is unknown. Although IgA is incapable of fixing complement or acting as an opsonin, the secretory IgA molecule together with complement and lysozyme, an enzyme found in all external secretions so far examined, is capable of killing *E. coli*.<sup>126</sup> The effects of IgA lack are described in Chapter 44. It is rather surprising that most deficient individuals appear to be rather healthy and not overly susceptible to upper respiratory infections. IgA antibodies against intrinsic factor have been reported in the gastric secretion of a patient with pernicious anemia.<sup>144</sup>

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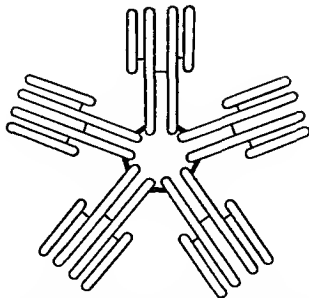


Fig 7-12. Tentative structure of IgM pentamer. See text for details

ence of genetic polymorphism of the  $\mu$ -chains remains to be established

In the intact IgM molecule five monomers are assembled in a star-shaped configuration, the carboxy-terminal ends (Fc pieces) being joined at the center through disulfide bonds, while the antigen binding sites (Fab pieces) extend toward the periphery (Fig. 7-12). The molecule appears to have a great deal of rotational freedom and, while bound to particulate antigen, may take on the appearance of a spider, its legs (Fab pieces) extending toward the plane of the antigen (eg, a cell surface) while its body, consisting of closely linked Fc fragments, protrudes from the center and is ideally suited for other functions, such as complement fixation (page 333).

Since the IgM molecule consists of five subunits, each with two antigen combining sites, 10 combining sites per IgM pentamer would be predicted. Usually this is indeed the case, especially when the antigen is relatively small.<sup>159</sup> However, with some antigens, only five combining sites per IgM molecule can be demonstrated, presumably because half the combining sites are blocked.<sup>139,159</sup>

A single J chain (page 311) has been found attached to the IgM pentamer by disulfide

bridges.<sup>157</sup> IgM monomers do not have J chains.

### *Biologic Properties of IgM*

Macroglobulins are restricted predominantly to the intravascular pool.<sup>159</sup> Little, if any, IgM crosses the placental barrier and most that is present at birth is of fetal origin.<sup>268</sup> Detectable levels of IgM may be synthesized by the human fetus as early as the twentieth week of gestation,<sup>140</sup> but high levels of IgM at birth are usually indicative of intrauterine sepsis.<sup>182</sup>

On cell surfaces a single molecule of IgM readily fixes complement (page 334), whereas antibody doublets are required for fixation by IgG.<sup>159</sup> However, although IgG is efficient at 4° and 37° C, IgM is very inefficient at the lower temperature.

Specific receptor sites on macrophages for the Fc region of IgM have been described in animal systems.<sup>154</sup> Such receptors may play a critical role in the process of phagocytosis of immune complexes.

Generally speaking, IgM antibodies are the first ones to be produced in a primary immune response (page 317), to be replaced

subsequently by IgG antibodies (page 317). In addition, however, certain types of antibody responses remain predominantly IgM, including those against lipopolysaccharide antigens such as the heterophil (Forssman) and Wassermann antibodies, isohemagglutinins, cold agglutinins, and antibodies to the O antigens of gram-negative bacteria.<sup>155</sup>

The role of macroglobulins in plasma cell dyscrasias is discussed in Chapters 52 and 53.

### *IgM Monomer*

Naturally occurring 7S to 8S IgM monomers have been identified in normal sera.<sup>159</sup> Higher concentrations of monomers also occur in various disease states, including systemic lupus erythematosus, Waldenstrom's macroglobulinemia (Chapter 53) and other hypergammaglobulinemic states, congenital rubella, and immune deficiency disorders such as ataxia telangiectasia and "dysgammaglobulinemia."<sup>130,153,179</sup> In some instances IgM monomers have been found to possess antibody activity against blood group substances<sup>183</sup> or cell nuclei ("antinuclear factors").<sup>175</sup> IgM monomers may be related to more primitive immunoglobulins and would appear to be synthesized as such, rather than to represent an *in vivo* or *in vitro* breakdown product of IgM.<sup>179</sup>

### *IgD*

This immunoglobulin is found in low concentration in normal serum (0.3 to 40 mg/dl),<sup>176</sup> and even in IgD myeloma the characteristic spike is frequently absent (Chapter 52). It consists of two heavy ( $\delta$ ) chains and two light ( $\kappa$  or  $\lambda$ ) chains, has a molecular weight of 180,000, and sediments at 7S.<sup>180</sup> It appears to be catabolized rapidly ( $t_{1/2} = 2.8$  days),<sup>174</sup> is confined largely to the intravascular space,<sup>174</sup> and does not cross the placental barrier.<sup>176</sup> Little is known of its fine molecular structure or its functional significance.

### *IgE*

Reaginic antibodies, which mediate acute and sometimes life-threatening allergic reactions in atopic patients, belong to this distinct immunoglobulin class.<sup>128,148</sup> IgE molecules also have two heavy ( $\epsilon$ ) chains and two light ( $\lambda$  or  $\kappa$ ) chains. They have a molecular weight of 200,000, a sedimentation coefficient of 8.2S, and contain 10.7% carbohydrate.<sup>148</sup> Their survival in the serum is shorter than that of any other immunoglobulin ( $t_{1/2} = 2.4$  days), and their serum concentration is in the range of 0.01 to 0.07 mg/dl, with a mean of 0.03 mg/dl.<sup>128</sup> Higher concentrations may be found in the sera of patients suffering from asthma, hayfever, eczema, the Wiskott-Aldrich syndrome (Chapter 44), and helminthic infections.<sup>128,148</sup> IgE-forming plasma cells are most frequent in the respiratory, gastric and intestinal mucosa and in the regional lymph nodes, but few are found in the spleen and in other lymph nodes.<sup>148</sup> Thus IgE, like IgA, is classified as a secretory immunoglobulin.

### *Biologic Properties of IgE<sup>128,148</sup>*

IgE antibodies are capable of sensitizing basophils and mast cells (Fig. 7-13). The Fc portion of the IgE molecule fits into specific receptor sites on the cell surface.<sup>149</sup> When bivalent or multivalent antigens bind to at least two adjacent IgE molecules, the mast cell is triggered to release vasoactive substances, especially histamine and the slow reacting substance of man,<sup>149,166</sup> which in turn are responsible for clinical manifestations such as wheal and flare reactions, bronchospasm, small vessel dilatation, and shock.<sup>149</sup> In addition to its nuisance function in allergic states, IgE may also play a part in normal body defense mechanisms (Chapter 44).

### *Induction of Antibody Synthesis*

ANTIGENS AND IMMUNOGENICITY.<sup>205</sup> Antigens are molecules capable of inducing an

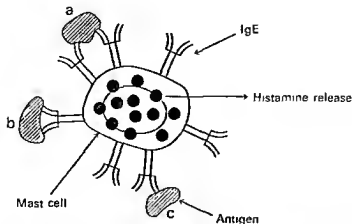


Fig 7-13 Schematic diagram of IgE mediated allergic reaction. Histamine release by mast cells is due to bridging of at least two cell-bound IgE antibodies by antigen, illustrated in (a), b and c cannot trigger histamine release

immune response. Haptens are molecules that are not inherently immunogenic, but that may elicit specific immune responses when they are bound to suitable carrier molecules, which must be immunogenic of their own accord. Hapten specific antibodies, once formed, are capable of combining with the hapten in the absence of the carrier. Haptens, on the other hand, are incapable of eliciting cellular immune phenomena (page 320).

The factors that control the immunogenicity of various antigens are incompletely understood, but several observations are clearly of importance: The ability to respond immunologically to some (if not all) antigens appears to be genetically determined.<sup>231</sup> This can be shown most clearly in animal models, but is undoubtedly also true for man. The genetically determined control mechanisms appear to be expressed at the level of T cell functions (page 316).<sup>232</sup> The physical state of the antigen is also important; the most powerful antigens such as bacteria, viruses, and cells are particulate, whereas soluble proteins are notoriously less effective antigens and are, indeed, highly suitable for the induction of immunologic tolerance (page 331).<sup>205</sup> Manipulations of antigens that increase their capture by the reticuloendothelial system, such as heat aggregation or alum precipitation, also increase antibody production.<sup>205</sup> This suggests that, for some antigens, the

interaction of antigen with macrophages or other phagocytic cells may constitute a necessary first step in the immune response (Fig. 7-14).

**FATE OF ANTIGEN AND ROLE OF MACROPHAGES.** Immunocompetent organs, such as the spleen and lymph nodes, process degradable antigens in two main ways.<sup>221, 236, 266</sup> The greater part of the material is phagocytosed by macrophages within the marginal zone or the medulla and is subjected to degradative enzymes within phagocytic vacuoles and lysosomes, while some is retained in unaltered form, especially at the cell surface. The smaller proportion of antigen is trapped in the lymphoid follicles of lymph nodes, spleen, and Peyer's patches, primarily on the surface of dendritic reticular cells, and is held there for prolonged periods of time. This process is greatly augmented in the presence of specific antibody.

There still is some controversy concerning the role of macrophages and other phagocytic cells in the induction of immunity. Generally speaking, as discussed elsewhere (Chapter 8) macrophages may subserve three types of functions<sup>266</sup>: (1) the removal of antigen from extracellular fluids, (2) the concentration and presentation of antigens to T and B cells, and (3) the change of antigen by degradative processes. The degree to which

these functions influence the immune response depends on many factors, including the type and amount of antigen and the anatomic site at which antigen arrives. For soluble antigens, for instance, macrophages are important for the removal of excess antigen which may lead to an ineffective immune response or tolerance,<sup>266</sup> and for presenting it in concentrated form to other cell types such as T and B lymphocytes.<sup>219,266</sup> For tissue or cell antigens, on the other hand, there is no need for antigen removal or concentration, since the antigen is already being presented in a readily accessible form and on a cell surface; lymphocytes can easily interact with such antigens in the absence of macrophages.<sup>266</sup> For particulate antigens such as bacteria and foreign red cells, both their removal and their concentration appear to be important while their partial degradation

may lead to an improved immune response.<sup>215,220,266</sup> Macrophages may also have the ability to form a highly immunogenic complex consisting of RNA and fragmented antigen ("superantigen") which is capable of inducing antibody formation in lymphoid cells.<sup>221,222</sup> Most of the evidence gives the RNA a carrier rather than an informational role.<sup>221</sup>

The binding of antigen to dendritic reticular cells appears to be enhanced by the presence of "opsonins" early in the primary immune response and by specific antibody subsequently.<sup>224</sup> The antigen is then transported from the outer cortex or marginal zone to the germinal centers by mechanisms that are not yet clear.<sup>224</sup> As lymphocytes move in and out of follicles they come into intimate contact with the deposited antigen and are triggered into blast transformation.<sup>236</sup>

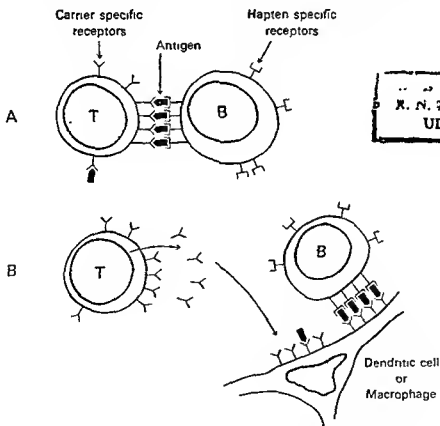


Fig. 7-14. Schematic representation of two mechanisms suggested for the interaction between T and B lymphocytes. A. Direct presentation of antigen by T cells to B cells. B. Specific antibody (IgT) is produced by T cells and is bound to macrophages or dendritic cells. B cells interact with antigen bound to "cytophagic" IgT on the surface of macrophages or dendritic cells. (Adapted from Feldman<sup>218</sup>)

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This mechanism may not play a part in the initiation of early primary immune responses characterized by IgM antibodies, but may act as part of the amplification system characteristic of the secondary (IgG) response.<sup>224</sup>

**THE ROLE OF LYMPHOID CELLS IN ANTIBODY PRODUCTION.** For most antigens the next step in the humoral immune response appears to require the active cooperation of at least two functionally different types of lymphoid cells.<sup>226,232,234,248</sup> The two co-operating cell types have been termed antigen reactive cells (ARC) and antibody forming cells (AFC). The antigen reactive cells are thymus derived (T cells) whereas the antibody producing cells are B cells. The exact nature of T and B cell interaction in antibody production is still incompletely understood, but several important factors have emerged from a large series of studies.<sup>219,226,232,234,243</sup>

(1) Both of these types of cells possess antigen specific receptors, the specificity of which is important in the cooperative effort of these cells. In the case of B cells, these receptors are unequivocally immunoglobulin in nature,<sup>226,239,243,248</sup> but in the case of T cells, evidence for the nature of such receptors has been difficult to obtain. Some studies suggest that these receptors may consist of classically defined immunoglobulin structures,<sup>204,240,250</sup> but it is also possible that they may consist of unique immunoglobulins (IgX) not found freely circulating,<sup>230</sup> or that they may be totally nonimmunoglobulin in nature.<sup>214</sup> Part of the difficulty may be due to the lower concentration of receptors on T cells ( $<10^3$ ) than on B cells ( $10^5$ ).<sup>267</sup> In addition to receptors for antigens, B cells have receptors for the Fc portion of immunoglobulins<sup>240</sup> and for complement.<sup>207</sup> Such receptors are apparently not demonstrable on T cells.

(2) It would appear that (processed) antigen first interacts with thymus derived antigen reactive cells, which are capable of responding to this antigenic stimulation by vigorous mitotic activity, but are incapable of antibody production.<sup>234</sup> In contrast, bone marrow cells, which are a convenient source

of B cells, do not by themselves respond to antigenic stimulation by mitosis. However, following interaction with sensitized T cells, B cells proliferate and differentiate into cells producing large quantities of antibodies.<sup>234</sup>

(3) Both T and B cells have immunologic specificity, but their cooperative effect in antibody production is at least in part due to the fact that they subserve different orders of specificity. This is most clearly evident in the case of antigens that have well-defined carrier and haptenic groups. Thus B cells, which produce antibodies reacting with relatively small determinant groups, appear to interact primarily with haptens, whereas T cells have specificity for aspects of the carrier molecule.<sup>226,243</sup> It has also been shown that effective cooperation between T and B cells occurs only if the hapten and carrier are linked, i.e. are present on the same molecule.<sup>235</sup> These experiments have led to the suggestion that the antigen forms a bridge between T cells, which recognize carrier determinants, and B cells, which recognize and respond to the antibody inducing haptenic determinants. Thus T (helper) cells would serve to present the haptenic determinants to the antibody-producing cells (Figure 7-14).

(4) Under some conditions at least, direct contact between B cells and T cells may not be necessary.<sup>218,219</sup> Following stimulation with antigen *in vitro*, T cells may elaborate a molecule (IgT) that has the characteristics of an IgM monomer, binds antigen, and is strongly cytophilic for macrophages. IgT apparently binds to specific sites on the surface of macrophages, presumably via an Fc receptor, and presents to the passing B cells a carpet of antigen, held in place by the Fab pieces of the IgT molecule. In addition, T cells produce a number of nonspecific mediators which may augment proliferation of B cells and may influence the class of antibody secreted.<sup>218</sup>

(5) It must be noted that T and B cell cooperation is not invariable, since some antigens are capable of stimulating B cells directly. Such antigens are termed thymus independent and include pneumococcal polysaccharide, polymerized flagellin of *Sal-*

monella adelaide, E. coli polysaccharide and polyvinylpyrrolidone.<sup>226</sup> They usually consist of large polymeric molecules with identical repetitive determinants, whose structure may mimic the "carpet of antigen" on the surface of macrophages described previously. Generally speaking, thymus independent antigens only elicit IgM antibody responses.<sup>226</sup>

(6) In addition to their effect on the induction of antibody synthesis by B cells, stimulated T cells also influence several other areas of B cell function. These include the switch from IgM to IgG antibodies (see below), the change of antibody affinity with time,<sup>226,233</sup> and the activity of histocompatibility linked genes that determine immunologic responsiveness to certain antigens.<sup>231</sup> In addition, activated T cells may, under some conditions, be inhibitory rather than stimulatory and may therefore have a fundamental regulatory function in the immune response.<sup>226</sup>

### Antibody Synthesis

Following their interaction with macrophages and T cells (see above) antigen-stimulated B cells undergo a series of cell divisions which greatly increase the number of cells capable of producing specific antibodies.<sup>229</sup> Some of these cells differentiate into mature plasma cells which are highly specialized for antibody production but are no longer capable of division.<sup>209,229</sup> Eventually these cells die and have to be replaced by newly differentiated cells. This process requires the continuing presence of antigen.<sup>264</sup> If antigen is no longer available, the clon of proliferating cells will regress and antibody production will decrease.

Antibodies are synthesized in various lymphoid organs, depending to a large extent on the antigen's portal of entry<sup>264</sup>: in regional lymph nodes following intradermal or subcutaneous stimulation; in the spleen and sometimes in the bone marrow and the lung following intravenous injections; and in the subepithelial lymphoid tissues when antigen penetrates the gastrointestinal or respiratory mucosa.

### Heterogeneity of Antibody Production

Most antigens lead to the production of very heterogeneous populations of antibodies involving different classes of immunoglobulins, variations in affinity, and differences in specificity.<sup>255</sup>

In addition, all of these features may change with time, the direction of change depending in part on the type of antigen involved, the amount available, and whether the stimulus is repeated or not. In many instances, however, the changes follow predictable patterns.<sup>264</sup> In the case of particulate antigens, the *primary response*\* consists of 19S antibodies that are formed very early and may eliminate any remaining antigen. Serum antibody may appear as early as eight to 16 hours after intravenous immunization<sup>260,264</sup> but more commonly after two to four days. The concentration of antibody increases rapidly, and may double every six to 15 hours, depending in part on the dose of the antigen.<sup>264</sup> Thus 19S response generally peaks at about a week and is short-lived (Fig. 7-15). It is relatively resistant to radiation and 6-mercaptopurine but is sensitive to lymphocyte depletion procedures.<sup>264</sup> In comparison to particulate antigens, soluble antigens such as diphtheria toxoid lead to negligible or low concentrations of 19S antibodies.<sup>264</sup>

If the immunizing dose of antigen is sufficiently large, 7S antibodies may appear four to seven days after primary immunization; low doses of antigen, even when particulate, may result in 19S responses only. This primary 7S response may occur with particulate and with soluble antigen and is characterized by a slower rise in antibody titer (Fig. 7-15), but is of longer duration (months or even years). The 7S response is sensitive to x rays, especially before antigen is given, is inhibited by 6-mercaptopurine, and is sensitive to lymphocyte depletion procedures.<sup>264</sup>

If a second dose of antigen is given several weeks or months after the initial stimulus, a *secondary antibody response*\* is seen (Fig.

\* A *primary immune response* is one that results from the first encounter between an antigen and the immune system; the response that occurs after an appropriately spaced second encounter is referred to as a *secondary or anamnestic response*.



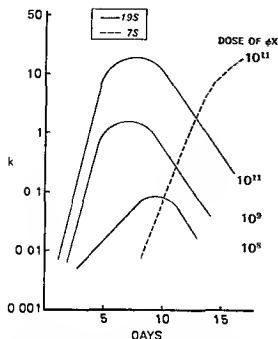


Fig 7-15 Primary ( $19S$ ) and  $7S$  immune responses to the phage  $\phi X$  in the guinea pig. Representative responses to injections of  $10^{11}$ ,  $10^9$ , or  $10^8$   $\phi X$  are shown. A  $7S$  response is detected only with larger doses of  $\phi X$  (From Uhr and Finkelstein<sup>264</sup> courtesy of the authors and *Progress in Allergy*.)

7-16)<sup>264</sup> The secondary response can be elicited by very low concentrations of antigen and the latent period for antibody formation is shorter than after the primary stimulus; the initial rise in antibody titers is exponential, and consists almost exclusively of  $7S$  antibodies. After reaching a peak, antibody levels drop rapidly at first, but then reach a plateau which persists for long periods of time. The secondary response is radioresistant, 6-mercaptopurine insensitive, and not affected by lymphocyte depletion maneuvers.

The term "immunologic memory" is used to describe the capacity to produce a secondary antibody response.<sup>264</sup> Immunologic memory must of necessity be a function of long-lived cells. Earlier experiments attributed memory entirely to the long-lived  $T$  cells,<sup>11,13</sup> but it is now known that both  $T$  cells and some  $B$  cells are capable of expressing specific immunologic memory.<sup>226,233</sup> Such  $B$  cells pre-

sumably form part of the pool of long-lived recirculating cells and are unlike the short-lived  $B$  cells described previously (page 299).

### Functional Heterogeneity of Antibodies<sup>217,256</sup>

Anti-2,4-dinitrophenol antibodies obtained from one bleeding of an individual rabbit have been shown to contain fractions with 10,000-fold differences in binding affinity.<sup>217</sup> The average affinity of specific antibodies tends to increase progressively with time after immunization, a phenomenon termed "maturation of the immune response,"<sup>255</sup> perhaps reflecting selection of cells with higher and higher affinity receptors as the concentration of available antigen drops progressively with time.<sup>255</sup> Cells with high affinity receptors would in turn elaborate antibodies of equally high affinity. From a clinical point of view the maturation of antibody affinity is probably more important than the amount of antibody produced. This is due to the wide range of affinities encountered and the importance of affinity in biologic systems.<sup>225,256</sup> In addition, many *in vivo* immune phenomena offering protection to their host operate at low antigen concentrations. In this situation high affinity antibodies may offer a crucial advantage.

The factors that favor maturation of the immune response include<sup>255</sup> (1) optimum doses of antigen, preferably given as a depot; optimal doses need to be determined for each antigen, but in most clinical situations too much rather than too little has been used, (2) the physical state of the antigen, since aggregated antigens are more immunogenic whereas soluble antigens may render high affinity cells selectively unresponsive; (3) the use of immunologic adjuvants.<sup>200,201</sup> Detailed reviews have dealt with other factors that control antibody synthesis such as genetic factors,<sup>231</sup> antigenic competition,<sup>263</sup> the role of circulating antibody,<sup>264,265</sup> and the effect of  $T$  cells.<sup>226</sup>

### Molecular Aspects of Antibody Biosynthesis

Knowledge regarding the biosynthesis, assembly, and secretion of immunoglobulins has been derived from studies involving normal lymphoid organs, mouse plasmacytomas, human tumors, and cell-free systems.<sup>139,209</sup> The DNA sequences coding for heavy and light chain structures are probably located on different chromosomes, since there is no evidence for linkage between them.<sup>221</sup> The coding sequence and consequent production of ribosomal immunoglobulin are similar or identical to those of other proteins (Chapter 2). In rats and mice the bulk of antibody appears to be made on membrane bound

ribosomes,<sup>268</sup> whereas in at least one human lymphoid line only the L chains are synthesized on membrane bound ribosomes, the H chains being made on free cytoplasmic polyosomes.<sup>254,259</sup> It is likely that immunoglobulin can be synthesized on either free or membrane bound polysomes and that the site of synthesis is determined largely by the availability of a well-developed rough endoplasmic reticulum. This is characteristically present in plasma cells but to a lesser extent in lymphoid cells (page 289).

Heavy and light chains are synthesized separately, the heavy chains on large 270S to 300S polyribosomes composed of 16 to 20 subunits, the light chains on smaller 190S to 200S polyribosomes composed of seven to

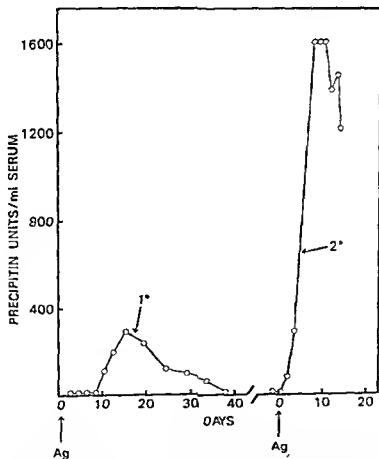


Fig. 7-16. Primary (1°) and secondary (2°) antibody responses of rabbits injected once (1°) and twice (2°) with an interval of four weeks between injections (From Dean and Webb, *J Path Bact* 31:89, 1928, as illustrated in *Microbiology*, David et al<sup>214</sup> eds, courtesy of the authors and Hoeber Medical Division, Harper and Row)

eight subunits.<sup>139,209</sup> The size of these poly-somes is such as to suggest synthesis of each chain as a single unit.<sup>139</sup> Under normal conditions, light chains may be synthesized in slight excess.<sup>202,253</sup>

Following separate synthesis of heavy and light chains there may be some assembly of free light chains and ribosome bound heavy chain, but most assembly appears to occur after the release of polypeptide chains from the ribosomes; this may result in the formation of H-L half molecules initially, two of which then combine to form a complete gamma globulin monomer. Intermediate H<sub>2</sub>L and H<sub>2</sub> structures have, however, also been isolated, suggesting that the final H<sub>2</sub>L<sub>2</sub> structure may be reached by several pathways.<sup>209</sup> In most instances polymeric gamma globulins such as IgM (9S) and IgA (9S, 11S, 13S) are assembled intracellularly from their constituent subunits,<sup>209,210</sup> but a few studies suggest assembly at the time of secretion or extracellularly.<sup>211,257</sup> J chain has been detected intracellularly, bound to IgM or IgA polymers.<sup>212</sup>

The attachment of carbohydrate probably begins on the ribosome but, except for glucosamine, is not complete until the protein is released into the cytoplasm.<sup>252,254</sup> The complete or nearly complete immunoglobulin molecule passes to the Golgi zone. There the immunoglobulin molecule attaches to membrane and additional monosaccharides are attached with the aid of appropriate enzymes, so as to form polysaccharide chains. The sugars added include additional glucosamine, galactose, and, finally, sialic acid.

It has been suggested that the carbohydrate moiety facilitates the secretion of immunoglobulins by the cell,<sup>261</sup> but this aspect of antibody production is poorly understood.

Although the synthesis of light chains and heavy chains takes only 30 and 60 seconds, respectively,<sup>139</sup> the addition of carbohydrates and the process of secretion take at least half an hour. Approximately 10<sup>13</sup> molecules (2.2 g) of IgG are synthesized per second in an adult, plus an equal number of other immunoglobulins.<sup>189,258</sup> The distribution and fate of individual immunoglobulins have been discussed previously (Table 7-3).

The vast majority of antibody synthesizing cells contain only one type of heavy chain and one type of light chain,<sup>206,212,213</sup> but a small number of cells (usually less than 1%) contain more than one type of heavy chain, usually  $\mu$  and  $\gamma$ .<sup>213,244</sup> This observation suggests that a single cell might switch from IgM to IgG synthesis during the course of the immune response.<sup>237</sup>

### Cellular Immunity

In addition to their important contribution to antibody production (page 316), thymus derived lymphocytes (T cells) also play a central role in the induction and execution of cellular immunity. Cellular immune phenomena are those that result from the interaction of antigen with sensitized lymphocytes, rather than with humoral antibody. Characteristically, such responses can be transferred passively from a sensitized individual to a nonsensitized one by cells but not by circulating antibody.

Most reactions of cellular immunity develop more slowly than those mediated by antibody, hence the term "*delayed hypersensitivity reaction*" which, in addition to its specific meaning (page 325), frequently is used in a generic sense to include all cellular immune phenomena. Clinically important examples of cellular immunity are listed in Table 7-5.

### Induction of Cellular Immunity

The immunogenicity of antigens and their handling by macrophages are as important in the induction of cellular immunity as in the

**Table 7-5. Clinical Expressions of Cellular Immunity**

- 
- 1 Cutaneous delayed hypersensitivity
  - 2 Contact allergy
  - 3 Immunity to intracellular parasites
    - a Facultative intracellular bacteria
    - b Viruses
    - c Protozoa
    - d Fungi
  - 4 Allograft rejection
  - 5 Graft versus-host disease
  - 6 Tumor immunity and immunologic surveillance
  - 7 Autoimmune diseases
-

induction of antibody synthesis. Several factors favor the induction of cellular immunity, including (1) immunization by the intradermal or subcutaneous, rather than the intravenous route<sup>374</sup>; (2) the incorporation of antigen into immunologic adjuvants, which usually contain killed tubercle bacilli in mineral oil<sup>264,312</sup>; and (3), in natural infections, the type of organism involved; generally speaking, intracellular parasites such as mycobacteria, salmonella, brucella, and viruses induce delayed hypersensitivity most readily.<sup>377</sup>

As in the induction of antibody synthesis, macrophage bound or processed antigen is capable of interacting with antigen specific T cells,<sup>266,375</sup> presumably in the paracortical areas of lymph nodes and, to a lesser extent, in the thymus dependent areas of the spleen. In vitro experiments have shown that close contact between macrophages and lymphocytes is essential<sup>294</sup> and may be facilitated by the presence of uropods on T cells (page 287). T cells react with antigen by virtue of specific receptors for single antigenic determinants.<sup>214,251</sup> These small lymphocytes transform into blast-like forms, undergo clonal proliferation, and produce specifically sensitized small lymphocytes.<sup>13</sup> Morphologically this event is reflected in the appearance of pyroninophilic cells in the paracortical areas of draining lymph nodes.<sup>13,20</sup> Lymphocytes not coming into direct contact with antigen may be recruited into the immune response by the release of humoral mediators, such as transfer factor (page 324) and other blastogenic factors produced by sensitized cells (page 323). Some studies suggest that T-T cell interaction occurs during the development of cellular immunity,<sup>226</sup> just as B-T cell interaction is essential for the induction of antibody production (page 316). Within a few days following antigenic stimulation, blast-like cells are seen within the efferent lymph of stimulated nodes.<sup>325,326,327</sup> These cells may migrate to other lymphoid tissues<sup>327</sup> and thereby lead to systemic sensitization.<sup>309</sup> They also act as effector cells in cell-mediated immune responses (see below) and carry immunologic memory<sup>317</sup> (page 318).

## Mechanisms of Cellular Immunity

The properties of sensitized T-cells that are important in the effector arc of cellular immunity (Table 7-4) include the ability to recognize and interact with specific antigenic determinants, the ability to respond to this interaction by further proliferation, the production of soluble mediators of immunity, and the capacity to kill antigen-bearing target cells on contact.

The recognition of antigen by sensitized T cells ("memory cells") is mediated by highly specific receptors,<sup>243,300,383</sup> presumably similar to those found on antigen reactive cells (ARC's) in unprimed individuals (page 316). Under in vitro conditions, antigen-lymphocyte interaction results in transformation and clonal proliferation of sensitized cells.<sup>348</sup> In most instances, lymphocyte transformation correlates with delayed-type hypersensitivity in vivo,<sup>351,354</sup> but exceptions have been noted.<sup>285</sup> In vitro lymphocyte stimulation has illuminated many aspects of cellular immunity, including the need for macrophages in the induction of proliferation in sensitized cells,<sup>283</sup> the high degree of carrier specificity of these reactions,<sup>243,364</sup> and the heterogeneity of cellular immune responses to antigen in vitro, which probably reflects a range of binding affinities of cellular receptors for antigen comparable to that seen in secreted antibodies (page 318).<sup>235,335</sup>

## Humoral Mediators of Cellular Immunity

When sensitized lymphocytes react with antigen in vitro, they elaborate into the culture medium a number of biologically active substances.<sup>296</sup> Many of these substances (Table 7-6) are capable of eliciting inflammatory reactions and, since only a few sensitized lymphocytes can produce enough of these mediators to affect a relatively large number of cells, the whole reaction is amplified greatly.<sup>299</sup> The properties of a number of these substances, derived from supernates of antigen-sensitized lymphocyte cultures and sometimes referred to as *lymphokines*, will be summarized briefly.

**Table 7-6. Humoral Mediators of Immunity**

- 
- 1 Mediators affecting macrophages
    - a Migration inhibitory factor (MIF)
    - b Macrophage activating factor
    - c Macrophage chemotactic factor
  - 2 Chemotactic factors for
    - a Neutrophils
    - b Eosinophils
    - c Lymphocytes
  - 3 Toxic and growth inhibitory factors
    - a Lymphotoxin
    - b Cloning inhibitory factor
    - c Proliferation inhibitory factor
    - d Inhibitor of DNA synthesis
  - 4 Blastogenic or mitogenic factors
  - 5 Skin reactive factors
  - 6 Immunoglobulin
  - 7 Interferon
  - 8 Transfer factor
- 

***Migration Inhibitory Factor (MIF)***

MIF inhibits the migration of normal macrophages out of capillary tubes (Fig. 7-17)<sup>285,296</sup> Although the production of MIF is immunologically specific, the inhibition of macrophages occurs in a random and nonspecific manner, even in the absence of antigen. MIF is a nondialyzable macromolecule with a molecular weight of 35,000 to 55,000; it is a nonimmunoglobulin acidic glycoprotein which is destroyed by chymotrypsin. Its biologic effect is also destroyed by neuraminidase, suggesting that sialic acid residues are necessary for its activity.

***Macrophage Chemotactic Factors (MCF)***<sup>379</sup>

MCF can be differentiated from MIF on the basis of their distinct physicochemical properties. Separate chemotactic factors for lymphocytes, neutrophils, and eosinophils also have been identified,<sup>297</sup> the one for eosinophils requiring antigen-antibody complexes for its elicitation.

***Macrophage Activating Factor***

By present techniques this factor is indistinguishable from MIF.<sup>296</sup> It is produced by sensitized and stimulated T cells and leads

to the "activation" of macrophages.<sup>345</sup> Activated macrophages are larger and more complex morphologically than are ordinary ones. They also have a marked propensity to spread on glass and are more avidly phagocytic. Activated macrophages have an increased content of acid hydrolases, an increased digestive capacity, and an increased mitotic rate. Thus activated macrophages acquire a greater ability to deal with intracellular bacteria and other phagocytosable matter.

Although release of macrophage activating factor is triggered by immunologically specific events, the consequent macrophage activation results in heightened cellular (macrophage-mediated) immunity towards a variety of microorganisms as well as noncellular antigens.<sup>315</sup> Thus, as for MIF, the production of macrophage activating factor is immunologically specific, but its effect is nonspecific.

It is readily apparent how MIF, the chemotactic factor, and the macrophage activating factor might collaborate at an inflammatory site. A specifically sensitized lymphocyte interacts with an appropriate antigen, eg, bacterial, and elaborates all three principles; the chemotactic factor attracts macrophages to the area, MIF confines them to the inflammatory site, while the macrophage activating factor induces an increased capacity to deal with the invading organism.

***Lymphotoxin (LT)***

Cell-free supernatants from lymphocyte cultures stimulated by specific antigen or nonspecific mitogens, such as phytohemagglutinin (page 341) or antilymphocyte serum, contain a substance that is cytotoxic for a variety of target cells in vitro.<sup>318,357</sup> Similar cytotoxic factors are produced by sensitized lymphocytes when stimulated by living or dead viruses,<sup>285</sup> as well as by long-term lymphocyte cultures in the absence of stimulation by antigen or mitogens.<sup>285</sup> Human lymphotoxin is a nonimmunoglobulin protein migrating with the  $\beta$  and  $\gamma$  globulins.<sup>285</sup> It has a molecular mass of 80,000 daltons and is inactivated by heating at 80° C.<sup>318</sup> Its production is inhibited by a variety of metabolic

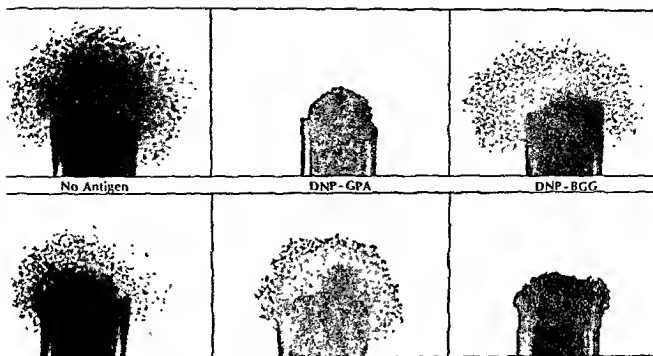


Fig. 7-17. Quantitative assays of migratory inhibition of macrophages by MIF. The migration of cells from an animal sensitized to DNP-GPA is inhibited only by the DNP-GPA antigen and not by DNP conjugated with another carrier protein (DNP-BGG). With animals sensitized to DNP-BGG, MIF activity is similarly specific, in this instance DNP-GPA produces a "control" result (from David<sup>298</sup> courtesy of the author and Sinauer Associates, Inc.)

inhibitors but its release is probably not closely linked to DNA synthesis.<sup>355</sup>

The cytolytic activity of lymphotoxin is readily demonstrable *in vitro*. There is a wide range of sensitivity to its action among various cells, human lymphocytes being the most resistant and mouse L cells the most sensitive.<sup>285</sup> The amount of lymphotoxin produced *in vivo* may not be sufficient to permit a cytotoxic effect on target cells, however. Its primary *in vivo* effect may be growth inhibition.<sup>285</sup>

Other growth inhibiting factors have been described in supernatants of antigen stimulated cells, including the "cloning inhibitory factor,"<sup>285</sup> the "proliferation inhibitory factor,"<sup>321</sup> and the "inhibitor of DNA synthesis" (IDS).<sup>357</sup>

### Blastogenic Factors

In addition to factors inhibiting growth, various factors that have a stimulatory effect on other lymphocytes are also produced.

They include factors capable of stimulating other lymphocytes in the absence of antigens,<sup>311,359</sup> those requiring antigen,<sup>336,376</sup> and some that allow purified lymphocytes to be stimulated by antigen in the absence of macrophages.<sup>278</sup> Other blastogenic factors make normal lymphocytes cytotoxic for target cells.<sup>311</sup>

### Skin Reactive Factors (SRF)<sup>285</sup>

SRF elicit reactions that resemble delayed-type hypersensitivity responses (page 325), but occur much earlier. The activity mediating this effect has not yet been distinguished from MIF, LT, or blastogenic factors.<sup>399</sup> A factor that results in skin lesions similar to those produced by SRF but that also contains an activity causing changes in vascular permeability has also been extracted from normal or sensitized lymph node cells. This factor has been termed "lymph node permeability factor" (LNPF).<sup>356</sup>

### Immunoglobulin

The synthesis of immunoglobulin by B cells was discussed in an earlier section (page 317). The synthesis of a  $\beta_2$  microglobulin by phytohemagglutinin stimulated lymphocyte cultures and long-term cultures has also been described.<sup>333,348</sup> In addition, it is possible that T cells may be capable of producing small quantities of an immunoglobulin-like factor which has been postulated to play a role in B-T cell interaction (page 316).

### Interferon

Interferons are proteins that confer antiviral resistance on cells that are normally virus susceptible. Agents that stimulate the production of interferon are known as inducers and include a large variety of substances<sup>323</sup> such as viruses; nucleic acids and especially double-stranded RNA; a variety of bacteria and their products including endotoxins and exotoxins; low molecular weight substances such as tilorone, cyclohexamine and kanamycin; and a number of substances that stimulate the proliferation of lymphocytes *in vitro*, including phytohemagglutinin, pokeweed mitogen, and specific antigens such as tuberculin. Although interferon may be produced by virtually all tissues of the body, lymphoid cells, especially T cells, appear to be a major source of interferon production.<sup>285,320,381</sup> Partially purified interferon preparations show a remarkable degree of structural heterogeneity with molecular weights ranging from 20,000 to 40,000 to over 100,000.<sup>323</sup> It has been suggested that human interferon may consist of multimeric aggregates of smaller subunits.<sup>289</sup> The monomer of virus-induced human interferon was found to have a molecular weight of 12,000. Interferon appears to be an unusually stable glycoprotein, but detailed structural studies are not available.

Following induction, the protein is synthesized and released quickly from the cell. This may occur as early as two to four hours after endotoxin, in six to 18 hours with viral in-

ducers,<sup>323</sup> and as late as four days when sensitized cells are stimulated by purified protein derivative (PPD) *in vitro*.<sup>283</sup> Interferon confers antiviral protection on the cell producing these proteins, but also carries the message of imminent danger to neighboring uninfected cells. It has been postulated that the antiviral activity of interferon in these cells is mediated by the synthesis of another protein<sup>314</sup> which, at the ribosomal level, interferes with the synthesis of virus coded enzymes and structural coat proteins necessary for viral replication.<sup>302</sup> Interferons display inhibitory activity against a large number of RNA and DNA viruses,<sup>323</sup> both cytotoxic and oncogenic, as well as a large number of nonviral infectious agents.<sup>323</sup>

### Transfer Factor (TF)<sup>343,347</sup>

In 1942 Landsteiner and Chase discovered that immune responses could be transferred from sensitive to nonsensitive individuals with live lymphoid cells.<sup>342</sup> In 1954, Lawrence demonstrated that delayed hypersensitivity could also be transferred by killed or disrupted cells.<sup>343</sup> Later it was shown that supernatants from specifically stimulated lymphocyte cultures were equally effective, but that cell populations so stimulated lost their ability to transfer responsiveness to the stimulating antigen but not to other sensitivities the patient carried.<sup>343</sup> Thus the sensitizing factor is antigen specific and is not immediately replenished. This curious immunologic phenomenon is mediated by a small molecule, appropriately termed *transfer factor*.<sup>343</sup> It is readily dialyzed and has a molecular weight of less than 10,000. Transfer factor is nonantigenic, nonimmunoglobulin, and relatively heat labile, being destroyed at 56° C for 30 minutes. It appears to be of polypeptide and/or polynucleotide composition but its detailed structure is unknown.

The immunologic sensitivities conferred on the recipient and his circulating lymphocytes are always concordant with those possessed by the donor of transfer factor. Sensitivity to a number of bacterial antigens, fungal antigens, and histocompatibility anti-

gens has been transferred successfully, always with a great degree of immunologic specificity. Results of skin tests are positive to specific antigens and the recipient's lymphocytes respond to antigen *in vitro*. Such sensitivity may last for months or years and may even be transferred from the recipient to other insensitive donors.<sup>343</sup> Transfer factor does not induce antibody production.<sup>343</sup>

It has been postulated that transfer factor may represent a convenient way of rapidly augmenting the number of cells capable of reacting with a specific antigen.

### Lymphocyte Cytotoxicity

Evidence for the ability of lymphocytes to cause tissue damage directly comes from at least four different *in vitro* situations in which target cell destruction could be demonstrated.<sup>285,356</sup> (1) Lymphocytes sensitized *in vivo* or *in vitro* to specific target cell antigens may be cytotoxic for these cells in the absence of demonstrable antibody or complement.<sup>287,292,356,362</sup> (2) Sensitized lymphocytes stimulated by specific antigen, or non-sensitized lymphocytes stimulated by nonspecific mitogens, may exert cytotoxicity on target cells antigenically unrelated to the stimulating agent.<sup>285,356,363</sup> (3) Lymphocytes from nonsensitized individuals may be induced to cause cytolysis of target cells by the presence of antitarget cell antibodies, bound to the target cell.<sup>285,356</sup> (4) Normal lymphoid cells may lyse target cells coated with certain complement components.<sup>356</sup>

The mechanisms of lymphocyte mediated toxicity are poorly understood, but several important principles have been established.<sup>285,356</sup> (1) Although cytotoxicity is mediated by lymphocytes, macrophages and granulocytes may contribute to the killing of target cells. (2) When the reaction involves lymphocytes sensitized to specific surface antigens of target cells, the cytotoxic reaction is exquisitely specific for cells carrying the immunizing antigen, other cells being unaffected. In all other situations, and especially when lymphocytes are activated by unrelated antigens or mitogens, the lymphocytes be-

come nonspecifically cytotoxic for a variety of syngeneic, allogeneic, and xenogeneic target cells. (3) Intimate contact between lymphocyte and target cell is required but this does not exclude the possibility that cytotoxic factors may be produced locally. (4) Lymphocytes have to be alive and metabolically active in order to express cytotoxicity. (5) The correlation between delayed hypersensitivity reactions *in vivo* and specific cytotoxicity for target cells *in vitro* is often excellent, but this does not exclude the participation of other cells from reactions of cytotoxicity.<sup>322,341</sup> (6) In some *in vitro* systems lymphotoxin (page 322) appears to play a crucial role in mediating lymphocyte cytotoxicity, since anti-lymphotoxin antibodies effectively prevent cytotoxicity.<sup>378</sup>

At the present time, *in vitro* models of lymphocyte mediated cytotoxicity provide the most important clues for interpreting a variety of immunologic phenomena of clinical interest, including those of tumor immunity, allograft rejection, and some autoimmune and other phenomena, but much more needs to be learned. Indeed, some mechanisms found *in vitro* may have no corresponding counterpart *in vivo*.<sup>283</sup>

### Clinical Expressions of Cellular Immunity

#### Delayed Hypersensitivity Reactions<sup>282,286,297,374</sup>

When antigens like tuberculin are injected into the skin of sensitized individuals, an area of erythema and induration appears after a lag of several hours and reaches maximal intensity in 24 to 72 hours. Microscopically, this lesion initially is characterized by capillary dilatation and exudation of fluid; by four to six hours a cellular infiltrate is seen which at first consists of polymorphonuclear granulocytes, but by 24 hours these are almost totally replaced by mononuclear cells.

The generation of such cellular infiltrates requires two types of cells: sensitized lymphocytes and macrophages. The initiating event is a reaction between specifically sensi-



tized lymphocytes and antigen that results in the release of a number of soluble mediators. Perhaps the most important of these are macrophage chemotactic factors (page 322) and, especially, MIF (page 322). These factors attract macrophages to the area and immobilize them at the injection site. In a fully developed delayed hypersensitivity reaction at least 95% of the accumulated mononuclear cells are macrophages, and less than 5% of the cells consist of specifically sensitized lymphocytes. The specifically committed lymphocytes accumulating within inflammatory exudates may also be derived from a rapidly dividing population of cells that seems to have a particular propensity for localizing in inflammatory foci.<sup>339</sup> Long-lived lymphocytes appear to be excluded from such areas.<sup>339</sup>

MIF or a closely related factor is also responsible for the activation of trapped macrophages (page 322). These activated macrophages are primarily responsible for the inflammatory features of delayed hypersensitivity reactions, although other factors such as lymphotoxins (page 322) and skin reactive factors (page 323) may play a minor role. Still other factors such as transfer factor (page 324) may serve to amplify weak local reactions by recruiting uncommitted lymphocytes into the pool of specifically sensitized cells, while some of the blastogenic factors (page 323) may aid in their recruitment and proliferation, but this remains to be established.

*Allergic (eczematous) contact dermatitis* is a clinically important example of delayed hypersensitivity reactions. Causal agents are generally small molecular weight compounds such as dyes, cosmetics, topical medications, industrial substances, plastics, and allergenic plant extracts such as those obtained from poison ivy (pentadecylcatechol).<sup>280</sup> Application of one of these substances to the skin of susceptible individuals can lead to sensitization so that a second application of the same chemical a week or more later produces a delayed inflammatory response with all the morphologic characteristics of delayed hypersensitivity reactions.

The mechanisms of sensitization have been elucidated by the use of synthetic contact sensitizers such as dinitrochlorobenzene (DNCB).<sup>306,308,503</sup> DNCB behaves as a true hapten (page 314), being incapable of causing sensitization unless firmly coupled to an immunogenic carrier molecule. The coupling is provided by a variety of proteins within the upper half of the epidermis, to which DNCB residues bind by covalent bonds.<sup>308</sup> Presumably covalent bonding of haptens distorts carrier proteins sufficiently to render them unrecognizable as self and therefore potentially immunogenic. Sensitization is generally dependent on adequate lymphatic drainage from the site of application to the regional nodes.<sup>313</sup> The processes within nodes are similar to those described for other types of cellular immunity (page 321). Sensitized cells then disseminate to other parts of the lymphatic system.

In order to elicit a delayed hypersensitivity response after sensitization, the hapten must again be conjugated with a carrier substance.<sup>307</sup> Such secondary reactions are most readily elicited in skin, but may also occur in mucous membranes.<sup>280</sup> The high degree of carrier specificity of delayed hypersensitivity reactions<sup>28a</sup> requires that structurally similar hapten-protein conjugates be formed on second exposure to the hapten. Contact sensitivity may also be transferred from one individual to another by means of lymphocytes,<sup>291</sup> suggesting that similar families of conjugates are produced by different patients.

### Role of Cellular Immunity in Infection

Cellular immune responses, of which delayed hypersensitivity reactions are a prototype, are of paramount importance in the defense against a number of obligate or facultative intracellular parasites such as viruses, rickettsiae, mycobacteria, *L. monocytogenes*, *Brucella abortus*, *S. typhosa*, and certain protozoa.<sup>345</sup> It is significant that, although secretory or circulating antibodies are capable of inactivating some infectious agents such as viruses and plasmodial parasites during cer-

tain stages of their life cycle,<sup>304,346</sup> and are capable of serving as opsonins for others, they leave many pathogenic bacteria and protozoan parasites quite unaffected, even during the extracellular phases of their existence. More importantly, antibodies and lymphocytes are completely incapable of dealing with infectious agents protectively accommodated within the cytoplasm of phagocytic cells.<sup>345,346</sup> Defense against such organisms obviously depends on measures that alter the intracellular environment from one in which the parasite normally prospers, to one that will not support its continued survival.<sup>346</sup> Such a mechanism is provided by the "activation of macrophages" by a product of sensitized lymphocytes (page 322).

The role of cell-mediated immunity in the control of viral infection is less clearly defined, although its importance is well established.<sup>297,377,382</sup> Lymphocytes do not normally cause virus neutralization,<sup>297</sup> but sensitized lymphocytes readily produce interferon following interaction with the virus (page 324). In addition, virus-infected cells carry new virus-induced antigens (page 329) and cell-mediated immunity may play a role in eliminating or destroying such cells.<sup>297</sup> It is also known that macrophages play a role in preventing the dissemination of virus,<sup>365</sup> and that macrophages from an immune host are more efficient than those from a non-immune one. Whether this increased efficiency is mediated by sensitized lymphocytes, increased interferon production, or some other intrinsic properties of macrophages is not known.

### Allograft Rejection<sup>13,387</sup>

Allograft\* rejection is an immunologic phenomenon, in most instances mediated by sensitized lymphocytes, rather than by circulating antibodies. The role of the latter will be discussed in a subsequent section (see below).

Sensitization is mediated by small recirculating lymphocytes, an unusually large num-

ber of which seem to be precommitted to interact with allogeneic histocompatibility or transplantation antigens.<sup>337</sup> A detailed discussion of human transplantation antigens is found in Chapter 12. Sensitization occurs within the graft (peripherally), possibly at the level of the graft's vascular endothelium,<sup>350,370</sup> or within lymph nodes ("centrally"), which have sequestered antigenic material from the bed of the graft.<sup>281</sup> Sensitization may also be a response to passenger cells, that is, host leukocytes carried over in the vessels of the allografts. Antigen-reactive cells triggered by contact with antigen then become established in thymus dependent areas of lymphoid tissue (page 295), and develop into large blast-like ("pyroninophilic") cells.<sup>301</sup> The proliferative response of these cells, possibly augmented by the recruitment of previously uncommitted cells (page 326), leads to the formation of a large clone of immunologically committed effector cells. This central proliferative response is indispensable to the generation of allograft immunity<sup>13</sup>; it is absent in animals incapable of responding because of effective immunosuppression, tolerance, or histocompatibility.

In man, cell-mediated allograft rejection usually occurs within one or two weeks after grafting.<sup>276</sup> The effector are that leads to the damage and destruction of solid tissue grafts is poorly understood. It appears to involve mononuclear cells, some of which are sensitized T cells, although many have a high mitotic index, even prior to the application of a graft.<sup>316</sup> Sensitized T cells ("killer cells") are capable of destroying graft cells on contact (page 325) without the need for complement or other serum factors,<sup>388</sup> but rejection may also involve the release of cytotoxic factors (page 322) or the biologic activity of activated macrophages (page 322), including the release of lysozymes within the vasculature of the graft.

The mixed leukocyte reaction (Chapter 12) is regarded as an *in vitro* correlate of allograft rejection *in vivo*.

Circulating antibodies may affect allografts in several ways: (1) When the recipient of solid organ grafts has high titers of antibodies

\* Allografts (or homografts) are tissue grafts transplanted from one individual to another of the same species.

directed at graft histocompatibility antigens, a "hyperacute rejection phenomenon" may be seen, usually within minutes of establishing vascular anastomoses.<sup>368,384</sup> This rejection occurs most commonly in kidney allograft recipients who have been presensitized by a prior graft, multiple blood transfusions, or multiple pregnancies. The early lesions are characterized by the accumulation of polymorphonuclear leukocytes and platelets (not lymphocytes) within the microvasculature, with progression to widespread capillary thromboses. Anti-HLA antibodies can be eluted from rejected kidneys<sup>384</sup> and may have triggered the entire reaction<sup>383</sup> which is morphologically similar to the Shwartzman reaction.<sup>368</sup> It has been suggested that high risk patients be treated prophylactically with anticoagulants,<sup>368</sup> but, although such therapy prolongs survival of canine renal allografts, it does not prevent their eventual rejection.<sup>275</sup> Others have attributed hyperacute rejection to intense vasospasm of unknown cause.<sup>303</sup>

(2) A patient in whom a renal allograft has been maintained for a prolonged period of time with the aid of immunosuppressive therapy may develop marked endothelial proliferation within the small vessels of the graft, thickening of the basement membrane, and fibrinoid necrosis of the vessel wall, which may be due to the chronic deposition of anti-allograft antibodies or to the deposition of antigen-antibody complexes along the basement membrane of the vessel walls.<sup>332</sup> Such a patient often develops the nephrotic syndrome. The reaction must be differentiated from the recurrence of renal disease, such as glomerulonephritis, which originally caused the patient's own kidneys to malfunction.<sup>332</sup>

(3) Under some circumstances non-cytopathic anti-graft antibodies may actually protect the graft against the activity of sensitized lymphocytes.<sup>332,359</sup> When such protection involves transplanted tumors, it is known as "immunologic enhancement."<sup>330</sup>

#### Graft-Versus-Host Disease (GvH)

Graft-versus-host disease refers to an immunologically specific phenomenon occur-

ring in an immunoincompetent host who is the recipient of viable, histoincompatible lymphocytes.<sup>284,319,349,366</sup> Most commonly such a reaction is seen in patients who have been rendered immunoincompetent by total body irradiation (in preparation for a bone marrow transplant) or in patients suffering from acquired or congenital immune deficiency syndromes (Chapter 44). In either instance, the patient is incapable of rejecting these histoincompatible cells which localize in lymphoid tissues and proliferate in response to the antigenic stimulation provided by the host's own tissues. In addition, the host's own lymph node cells are stimulated to proliferate extensively,<sup>284</sup> leading to lymphadenopathy and splenomegaly initially, but atrophy, fibrosis, and lymphopenia soon supervene. Other acute changes include evidence of tissue damage elsewhere: severe exfoliative dermatitis and hair loss, hepatitis, diarrhea and gastrointestinal ulcerations, severe wasting and emaciation, and a Coombs'-positive hemolytic anemia.

When the initial GvH reaction is mild, or when appropriate therapy with immunosuppressive drugs is given, the acute changes may regress and the patient (or experimental animal) may lead a relatively normal existence.<sup>315,329</sup> This regression appears to be due to the presence of blocking factors within the serum that prevent the GvH reaction.<sup>329</sup> In most instances, however, the patient succumbs to the acute manifestations of the underlying disease or to late complications, such as infections.<sup>349</sup>

Most of the manifestations of GvH disease, and certainly those that lead to fatal complications, are mediated by cells rather than by antibodies. It appears that interaction between distinct populations of thymus derived cells<sup>372</sup> and/or cooperation between thymus and bone marrow derived cells<sup>279,331</sup> is required for the expression of GvH reactivity.

When histoincompatible lymphocytes are injected under the renal capsule of nonirradiated hosts, a local GvH reaction results that appears to be due to the interaction of host and donor lymphocytes rather than to a direct attack by donor lymphocytes on kidney

cells.<sup>309</sup> Thus in some situations, at least, parenchymal cells may be damaged nonspecifically as "innocent bystanders."

### The Role of Lymphocytes in Autoimmune Diseases

The contribution of cellular immune phenomena to tissue damage in the so-called autoimmune diseases remains controversial. It has been suggested that autosensitized lymphocytes may play a role in the clinical manifestations of rheumatoid arthritis,<sup>328</sup> ulcerative colitis,<sup>350</sup> chronic liver disease,<sup>373</sup> Addison's disease,<sup>353</sup> and a number of neurologic diseases, including experimental allergic encephalomyelitis,<sup>277</sup> experimental allergic neuritis,<sup>277</sup> multiple sclerosis,<sup>290,360</sup> and the Guillain-Barré syndrome.<sup>338,360</sup>

### Antitumor Immunity

The concept of immunity against established tumors and the related concept of "immunologic surveillance" against emerging new clones of malignant cells<sup>407</sup> are based on two important hypotheses, namely, that tumor cells differ antigenically from normal cells and that host defense mechanisms are capable of recognizing and exploiting these differences. These hypotheses, first formulated with remarkable accuracy by Ehrlich in 1909,<sup>427</sup> have since been proved correct, both in animals and in man.<sup>410,421,422,425,427,433,440</sup>

Tumor specific transplantation antigens (TSTA or TSA), so named because their demonstration originally required transplantation techniques in syngeneic animal systems, are of two main types. Tumors induced by chemical carcinogens such as 3-methylcholanthrene<sup>410,434</sup> have tumor specific antigens that are distinct for each neoplasm. Even tumors induced in inbred strains and separate tumors induced in the same animal will all have different and readily distinguishable tumor antigens. In contrast, the tumor specific antigens of virus-induced tumors<sup>417,436</sup> are shared by all neoplasms caused by the same virus, even in different species. Such antigens are not present in the

virion or in normal cells before transformation. They represent, instead, structural alterations induced by the oncogenic virus within the host cells, probably on the basis of information contained within the viral genome.<sup>437</sup> Whether these changes represent newly synthesized antigens or uncovered subsurface antigens is not clear.<sup>406</sup>

When cells are infected with DNA oncogenic viruses, they may also produce other neoantigens associated with the nucleus or cytoplasm of the cell.<sup>403,437</sup> Known as T (transformation) antigens, they are, like TSA, identical on all tumors induced by a given virus. Their nature and function are unknown.

*Viral antigens* (Table 7-7) are rarely seen in DNA induced tumors but are regularly found in RNA induced tumors. Two types of viral antigens are recognized: (1) virus coat antigens and (2) antigens ordinarily confined to the viral core. Virus coat antigens are found on the cell surface, principally in areas of virus budding, whereas the cytoplasm contains both soluble coat antigens and virus core antigens. The latter include a number of viral proteins and enzymes, the best known of which is the RNA-dependent DNA polymerase.<sup>399</sup>

Tumor specific and structural viral antigens may be detected by a number of techniques which have been described elsewhere.<sup>410,437</sup>

Some antigenic materials produced by

**Table 7-7. Antigens of Virus-Induced Tumors**

<i>Antigen</i>		<i>DNA</i>	<i>RNA</i>
<b>A Structural (viron)</b>			
1	Cell surface viral envelope type specific	—	+
2	Cytoplasm internal structure group specific	—	+
<b>B Non-structural</b>			
1	Cell surface TSTA(S)	+	+
2	Nucleus Neo-antigen (T)	+	—

tumors are released from neoplastic cells into the circulation and body fluids. Such materials include  $\alpha$ -fetoprotein, found in the majority of individuals with hepatomas,<sup>410,413</sup> and carcinoembryonic antigen (CEA), found in the circulation of patients suffering from colonic carcinoma and other gastrointestinal neoplasms.<sup>410,414</sup> A closely related or identical antigen has been found in the sera of patients suffering from non-intestinal neoplasms (eg, breast, lung, prostate) or non-neoplastic disorders such as renal disease and alcoholic liver disease.<sup>410a,430a</sup> Different fetal proteins have been identified in other types of tumors.<sup>414</sup> It is also possible that soluble tumor specific antigens are shed into the circulation and body fluids where they may have an adverse effect on antitumor immunity (page 331).

*Immunity against tumor specific antigens* has been demonstrated by a variety of techniques, in both animals and man. Initially, tumor specific immunity was demonstrated by immunizing syngeneic animals against a tumor and subsequently challenging them with viable tumor cells.<sup>425,440</sup> Later, *in vitro* tests were developed for the detection of humoral and cellular antitumor immunity (Table 7-8). The colony inhibition test ap-

pears to be a particularly sensitive tool.<sup>419,422</sup> In this test, target tumor cells are incubated with antiserum or normal serum in the presence of complement and are plated on Petri dishes. The growth of colonies in antiserum treated cultures is compared to that in control cultures. When lymphocytes are used instead of antisera, the test can be used to measure cellular antitumor immunity.

Immunity against a large number of human neoplasms has now been established.<sup>422,423</sup> Such tumors include melanoma,<sup>430,431</sup> neuroblastoma,<sup>419</sup> Wilms' tumor,<sup>411</sup> choriocarcinoma,<sup>402</sup> and carcinomas of the colon,<sup>423</sup> breast,<sup>401,423</sup> lung,<sup>423</sup> ovary,<sup>423</sup> and bladder,<sup>405</sup> as well as several malignancies of special interest to hematologists, such as leukemia,<sup>416,435</sup> Hodgkin's disease,<sup>432</sup> and Burkitt's lymphoma.<sup>404,426,427</sup>

At this point it is relevant to ask whether the demonstration of antitumor immunity *in vitro* is of any consequence to tumor growth *in vivo*. Evidence for the importance of an intact system of antitumor immunity *in vivo* comes from a number of clinical observations and parallel *in vitro* studies: (1) Circumstantial evidence is derived from the observation that the incidence of malignancy is inordinately high in patients suffering from a

Table 7-8. Tests for Antitumor Immunity<sup>410</sup>

Technique	Source of Tumor Antigens	Immune Reagents	
		Type	Source
Precipitin (Duchterlony radioimmunoassay)	Soluble antigens from serum and body fluids	Antibody	(a) Sera from cancer patients, usually after excision or recovery (or from mothers in vertically transmitted tumors)
Fluorescent staining			(b) Immunized animals eg as a source of anti-CEA
Colony inhibition Cytolytic (target cell destruction)	Tumor cells	Antibody or lymphocytes	Cancer patients (or mothers in vertically transmitted tumors)
Lymphocyte transformation Macrophage migration inhibition	Tumor cells or soluble antigens	Lymphocytes	Cancer patients

variety of immune deficiency syndromes (Chapter 44), and in those subjected to chronic immunosuppression.<sup>415</sup> (2) There appears to be some correlation between prognosis and the overall immune status of the patient, both in leukemia (page 1397) and in other tumors.<sup>428,441</sup> (3) The most compelling evidence for a close link between immunity and malignancy comes from observations relating specific antitumor immunity to the prognosis of the patient. In several human tumors, especially malignant melanoma and colonic carcinoma,<sup>430</sup> the presence of antitumor antibodies in the serum is clearly associated with early or localized tumors, whereas dissemination of the tumor with metastases is associated with a lack of detectable antibody. Somewhat different results have been obtained with studies of cell-mediated antitumor immunity,<sup>420,422,423,437</sup> since there appears to be some discrepancy between the performance of the patient's lymphocytes *in vivo* (as judged by tumor progression) and the assessment of their capabilities *in vitro*. For, contrary to earlier expectations, there is no substantial difference between the *in vitro* antitumor activity of lymphocytes obtained from individuals in remission and from those with rapidly advancing tumors.<sup>423,437</sup> Thus patients with growing tumors do not suffer from a major breakdown of cell-mediated antitumor immunity. Instead, the tumors appear to be protected from the cytotoxic effects of specifically sensitized lymphocytes by blocking factors recoverable from the sera of patients with growing tumors. This blocking activity has the same immunologic specificity as the patient's immune lymphocytes and preliminary data indicate that it contains noncytotoxic 7S antibodies against tumor specific antigens.<sup>420,423</sup> Thus the protection of tumors by blocking sera bears some resemblance to the phenomenon of immunologic enhancement described in experimental tumor systems and allograft rejection (page 327): the tumor is protected against the cytolytic activity of sensitized lymphocytes because the surface bound tumor specific antigens are pre-empted by blocking antibodies.

In other experiments, blockade appears to be due to the presence of antigen-antibody

complexes in the serum.<sup>438,439</sup> The blockade is abolished when these complexes are dissociated, and is restored when the two parts are combined. These complexes may be capable of blocking lymphocytes by attachment to specific antigen receptors, whereas free antibody is restricted to blocking antigenic sites on tumor cells.

The sera of individuals in remission do not contain this blocking activity; its appearance may precede clinically detectable evidence of relapse.<sup>437</sup> Indeed sera of patients in remission appear to be capable of unblocking the sera of animals and patients with growing tumors.<sup>438,423</sup> In animals unblocking sera are capable of inducing tumor regression *in vivo*.<sup>423,437</sup> The implication of this finding for human immunotherapy needs to be explored.

The mechanisms of tumor rejection by lymphocytes are thought to be similar to those described for allograft rejection (page 327).

Undoubtedly, protection against malignant growth is not confined to lymphocytes and antibodies with specificity for tumor specific antigens. It is likely, for instance, that interferon plays a role in the protection against virus-induced malignancies.<sup>302,323</sup> Indeed, since oncogenic viruses are also immunosuppressive<sup>415</sup> and may therefore aid their own establishment in transformed cell lines, interferon may play an important protective role, especially in the early stages of tumor induction. The role of macrophages in antitumor immunity also needs further exploration. Destruction of tumors by macrophages from sensitized mice has been demonstrated both *in vivo* and *in vitro*.<sup>285</sup> It is possible that macrophages become cytotoxic by virtue of their ability to concentrate cytophilic antitumor antibody, or because of their activation (page 322) by products of sensitized lymphocytes.

#### Tolerance<sup>400,412,429,430b,413</sup>

Immunologic tolerance is an induced state of specific nonreactivity toward a substance that is ordinarily immunogenic. This type of specific tolerance depends on the interaction

between antigen and immunologically competent cells and is characterized by the subsequent failure of these cells to participate in the immune response. Tolerance is restricted to the antigen eliciting its induction and thereby differs from the nonspecific unresponsiveness induced by radiation, antilymphocyte serum, or drugs in the absence of antigen.

Other types of antigen induced immunologic unresponsiveness are due to interference with peripheral immune mechanisms rather than to the central paralysis of immunocompetent cells. These include the competitive inhibition of immune responses by haptens,<sup>430b</sup> soluble antigens, and antigen-antibody complexes (see blocking factors in antitumor immunity, page 331), as well as the phenomenon of antigenic competition.<sup>263</sup>

Whether immunization with a given antigen results in immune competence or tolerance depends to some extent on host factors, but, more importantly, on the nature of the inciting antigenic stimulus.<sup>430b,443</sup> *Host factors* favoring tolerance induction include immunologic immaturity, eg, during fetal development, and temporary but generalized states of immunosuppression due to radiotherapy or drugs, which are thought to mimic developmental immaturity. If antigen is introduced under these conditions, specific immune tolerance may result while the remainder of the immunologic apparatus develops or recovers normally.

**ANTIGENIC FACTORS.** In order to be tolerogenic (as opposed to immunogenic) antigen must reach all cells capable of reacting with it.<sup>413</sup> Thus small (monomeric) antigens that readily reach extravascular sites are more effective than are polymeric or aggregated antigens.<sup>412</sup> In addition, macrophages are less capable of fixing monomeric antigen,<sup>443</sup> and their important role in the induction of immune responsiveness (page 314) is thereby pre-empted. Instead, monomeric antigens may interact directly with specific immune responsive cells; this interaction may result in their removal or it may prevent their subsequent interaction with immunogenic forms of that antigen.

Tolerance may also be induced with very large ("high zone tolerance") or very small ("low zone tolerance") doses of antigen. High zone tolerance<sup>430b,443</sup> may be due to an exhaustive differentiation of cells specifically interacting with this antigen or to the direct access of antigen to lymphocytes without the participation of macrophages. The direct access theory has also been invoked to explain low zone tolerance,<sup>412,430b</sup> since macrophages bind antigens less efficiently than cells carrying specific antigen receptors; this gives lymphocytes a competitive advantage at very low concentrations of antigen. It also follows that cells with high affinity receptors should be more susceptible to tolerance induction than cells with low affinity receptors.<sup>442</sup>

Tolerance is not permanent and recovery may occur spontaneously or following a number of therapeutic maneuvers. *Spontaneous recovery* is affected by the persistence of antigen, the type of antigen, and age.<sup>412</sup> Thymectomy interferes with the recovery of immunologic reactivity.<sup>443</sup> Recovery may be induced by immunization with cross-reactive antigens and radiotherapy,<sup>430b,443</sup> the latter especially in partially tolerant states.<sup>443</sup>

Under most circumstances both T and B cells are rendered unresponsive,<sup>443</sup> although unresponsiveness of either component alone would affect antibody production. However, thymus derived (T) cells become unresponsive more readily than B cells and remain so for longer periods of time.<sup>443</sup> Macrophage function remains normal during tolerance.

**RELEVANCE TO DISEASE.** It has been suggested that the pathogenesis of some autoimmune disorders is related to a loss of tolerance for autoantigens, such as thyroglobulin in immune thyroiditis.<sup>413</sup> In other diseases, for instance rheumatic fever, tolerance may be "broken" by the production of antistreptococcal antibodies that cross-react with heart muscle fiber glycoproteins.<sup>424</sup> In addition, the widespread use of highly immunosuppressive drugs in the treatment of hematologic malignancies (Chapter 55) may foster the development of undesirable tolerance to a variety of determinants such as those of infectious agents and other tumor specific antigens.

## Complement<sup>446,447,468,480</sup>

The complement system consists of a group of 11 distinct serum proteins that interact sequentially to mediate certain effects of the inflammatory response. During the course of this reaction sequence the complement system also interacts with at least three other plasma protein systems, namely, the clotting system, the fibrinolytic system, and the kinin generating system.

### Nomenclature<sup>449</sup>

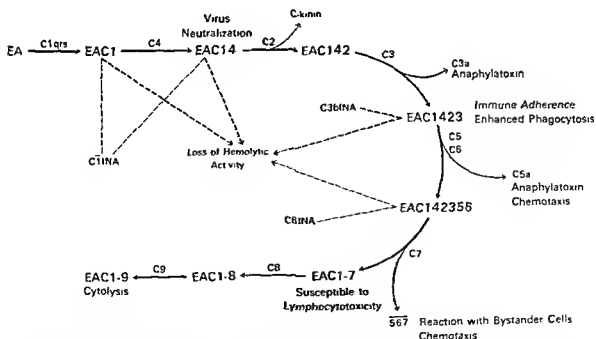
Most of our knowledge about the nature of the complement system has come from studies of sheep erythrocytes (E) treated with antibody (A) and complement (C). The various complement components interacting with the EA complex are indicated by arabic numerals, eg, C1, C3, etc. The first component, C1, consists of three subunits designated C1q, C1r, and C1s. Activated complement components in the fluid phase are indicated by a bar over the component number, eg,  $\bar{C}1$ . Activated components bound to cell surfaces are not so marked. Complement fragments

resulting from activation are suffixed sequentially with lower case letters, eg, C4a, C4b and the major hemolytically inactive fragments of each component receive the suffix "i", eg, C4i. Inactivators of specific complement components are indicated by the letters INA following the component in question, eg, C6INA.

Longstanding usage has led to the designation of the first four components in the reaction sequence as C1, C4, C2, and C3, in that order.<sup>448</sup> The remaining five components follow the more logical ascending numerical order.<sup>448</sup> The terms  $\beta 1E$ ,  $\beta 1C$ , and  $\beta 1F$  formerly designated the C4, C3, and C5 components, respectively.

### The Complement Cascade<sup>448,480</sup> (Fig. 7-18)

C1 is a complex molecule consisting of three proteins, C1q, C1r, and C1s, and calcium ions. The C1q subunit of C1 bears the combining site for the Fc portion of immunoglobulins and initiates the complement cascade by reacting with IgG or IgM antibodies that have combined with antigen or have been





aggregated by other means. The reaction requires either two IgG molecules that have reacted with antigen in critical proximity to each other, or two subunits ("monomers") of a single IgM molecule. Only IgG1, IgG2, and IgG3 antibodies are capable of activating complement, IgG4 is not (page 309). C1q then brings about the activation of C1r, which in turn converts C1s from a pro-esterase to an active esterase. This enzyme splits C4 into at least two portions, C4a and C4b, the larger of which, C4b, either becomes bound to the cell, forming EAC14, or remains in the fluid phase as the hemolytically inactive C4i. The smaller fragment (C4a, molecular weight 7,400) also appears free in the fluid phase. The interaction of C4 with C1 unmasks the capacity of the C1 enzyme to split its second natural substrate, C2, the major fragment (C2a) being bound to the cell surface in the presence of magnesium ions, thereby generating EAC142, a new enzymic activity, referred to as C3 convertase. This enzyme is very unstable and decays rapidly with the spontaneous release of the C2d fragment into the fluid phase.

Through the action of C3 convertase, C3 is split into a small fragment, C3a, and a larger fragment, C3b. The C3a fragment (anaphylatoxin) is released into the fluid phase. It causes a local wheal and flare reaction when injected intracutaneously in man, releases histamine from guinea pig mast cells in vitro, and causes the isolated guinea pig ileum to contract.<sup>455,465</sup> C3b is bound to form the cellular intermediate EAC1423 or remains free in the fluid phase as the inactive product C3i. The presence of C3b on the cell surface confers upon it the ability to participate in the phenomenon of "immune adherence"; this is a reaction in which C3b coated cells bind to specific receptor sites on platelets, polymorphonuclear leukocytes, and erythrocytes.<sup>462</sup> Binding of C3b coated cells to polymorphonuclear leukocytes is thought to enhance phagocytosis.

The C423 enzyme on EAC1423 cleaves C5 into C5a and C5b. The smaller C5a fragment is liberated into the fluid phase and has anaphylatoxin and chemotactic activity.<sup>458,491</sup>

The major fragment, C5b, is bound to the complex to form the EAC14235 intermediate which is quite unstable until the sequential binding of C6 and C7 has been completed.<sup>484</sup>

In the course of EAC1-7 formation, a factor that interacts with other bystander cells and is chemotactic for polymorphonuclear leukocytes is generated. This property is distinct from C5a and appears to be mediated by the trimolecular complex C567.<sup>492</sup>

The fixation of C8 to the EAC1-7 cell initiates membrane damage, but the rate of lysis is slow.<sup>493</sup> The addition of C9 markedly increases the rate of lysis. Cellular damage by human complement is associated with typical membrane changes, which on electron microscopic examination have the appearance of 10.3 nm holes.<sup>477</sup>

The biologic properties of complement are summarized in Table 7-9. Most complement components appear to be produced by macrophages with the possible exception of the intact C1 macromolecule which is either synthesized or assembled within intestinal tissue.<sup>480</sup>

**CONTROL MECHANISMS.**<sup>480</sup> Complement activity is limited by the presence of inhibitors and by the instability of some of its components, especially EAC142 and EAC14235. Inhibitors include C1INA, C3bINA, and C6INA.

**Table 7-9. Biologic Properties of Complement**

Complement Component	Biologic Activity
1 4	Virus neutralization
3b	Immune adherence Enhanced phagocytosis Arthus reaction
3a 5a	Anaphylatoxins Increased vascular permeability Arthus reaction
3a 5a 5 6 7	Chemotaxis of leukocytes Arthus reaction
1-9	Cell lysis Bactericidal reaction Transfusion reaction

### Alternate Pathways of Complement Activation<sup>169,480</sup>

Studies dealing with the activation of complement by zymosan, a polysaccharide extracted from yeast cell walls, lipopolysaccharide, and cobra venom factor (CoVF) have yielded incontrovertible evidence of an alternate pathway of complement activation that bypasses the activation of C1, C4, and C2.<sup>480</sup> In these alternate pathways the C3 activator system (see below) rather than C3 convertase (page 334) is responsible for the generation of C3b and C3a from C3, and the properdin system,<sup>472</sup> forgotten for over a decade, plays an important role.

*Properdin*<sup>470</sup> is a 5S  $\gamma_2$  glycoprotein of molecular weight  $180,000 \pm 12,000$  and seems to consist of four noncovalently bound subunits of 45,000 daltons each. It is an early reactant in the sequence leading to complement activation by the alternate pathway and requires at least three other constituents previously designated factors A, B, and D. *Factor A*, a hydrazine-sensitive euglobulin with

a molecular weight of 180,000, is in fact C3.<sup>469</sup> Factor B is a glycine rich, heat labile  $\beta$ -glycoprotein (GBG) which is identical to the C3 proactivator (C3PA) identified by others.<sup>469</sup> The third constituent is C3PA convertase (also called C3Pase, GBGase, or *factor D*), a euglobulin of 30,000 to 40,000 daltons; this factor converts inactive C3PA to the C3-cleaving enzyme, C3 activator (C3A).<sup>469</sup>

**PROPERDIN REACTION SEQUENCE.** Properdin is activated by inulin polysaccharides and other substances and reacts with C3 in such a way that it acquires C3Pase-activating capacity. This enzyme, in turn, utilizes C3PA as substrate, and the major product, C3A, then cleaves C3 to yield the C3a anaphylatoxin and C3b. C3b then initiates a positive feedback activation of C3Pase which is independent of properdin and activating substances such as inulin. This amplification loop is illustrated in the right hand panel of Figure 7-19. The C3b inactivator C3bINA exerts a controlling or damping influence on this feedback effect.<sup>477a</sup> When fixed on a cell sur-

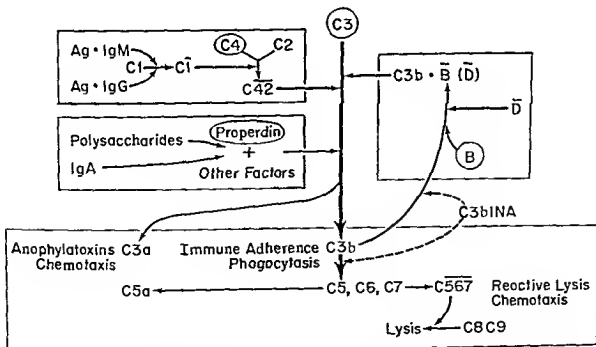


Fig 7-19. Interrelationships between the classic and alternate pathways of complement activation and the biologic activities associated with complement components 3 to 9. B = Factor B = C3PA, D = C3PA convertase (From Fearon et al.<sup>455a</sup> courtesy of the authors and *Advances in Nephrology*)

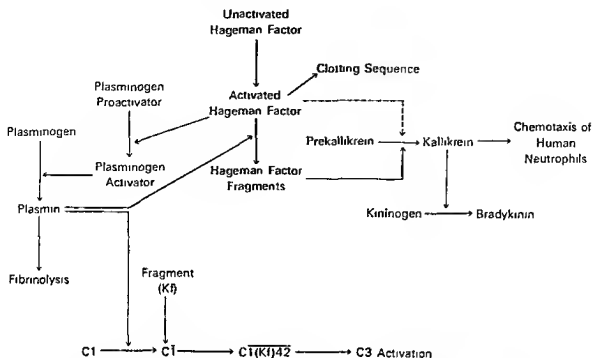


Fig 7-20 Interrelations between the coagulation, kinin generating, fibrinolytic and complement systems (From Ruddy et al.<sup>460</sup> courtesy of the authors and the New England Journal of Medicine)

face C3b can also engage the later C components, as discussed in the classic system (above). In addition, C3b can mediate the destruction of terminal C components in the fluid phase, and C3b itself can be inactivated by another protein called KAF.<sup>469</sup>

Alternate pathways are also activated by bacterial and fungal cell wall lipopolysaccharides, such as endotoxins,<sup>469,480</sup> and by cobra venom<sup>460,469,480</sup> and immunoglobulins.<sup>469</sup> Cobra venom factor (CoVF) forms a reversible complex with C3PA which has but feeble cleaving activity. C3PA convertase stabilizes this complex and enhances C3 cleaving activity. Another substance, *factor E*, is required for the enhancement of red cell lysis by this complex containing C3PA, CoF, and C3PA convertase. Immune activation of the alternate pathway ("C3 shunt") is mediated by sites found either on the Fc piece or on the F(ab)<sub>2</sub> fragment (page 306). Thus, aggregated F(ab)<sub>2</sub> fragments obtained from rabbit and guinea pig IgG as well as human IgA and IgE contain a site (near the hinge region) that is capable of activating complement via properdin and the alternate path-

way.<sup>490,469,482,483</sup> Reports concerning the ability of human IgG to activate the alternate pathway are conflicting.<sup>460,469</sup> Thus, immune activation via the alternate pathway provides a mechanism whereby IgA and IgE may activate complement and allows for the expression of antigenic specificity.<sup>480</sup>

#### Interaction of the Complement Sequence with Other Plasma Systems

The intricate entanglements of the clotting-fibrinolytic system, the complement sequence, and the kinin generating systems are illustrated in Figure 7-20.<sup>480</sup> (1) Activated factor XII (Chapter 10) initiates the clotting cascade and also activates the fibrinolytic system by the conversion of plasminogen to plasmin (Chapter 10). (2) Plasmin in turn digests activated factor XII with the production of active fragments which lead to kallikrein activation.<sup>463</sup> A limited degree of kallikrein activation is also mediated by activated factor XII. Kallikrein is directly leukotactic for human leukocytes and cleaves kininogen to yield vasoactive bradykinin.<sup>464</sup>

(3) The broad proteolytic activity of plasmin also includes the cleavage of C3 to yield anaphylatoxin (not shown)<sup>488</sup> and the activation of C1 to C1<sup>i</sup>.<sup>473</sup> In addition, the proteolysis of activated factor XII by plasmin leads to the activation of the kinin generating system, which in turn is associated with the appearance of a serum fragment, Kf,<sup>459</sup> that alters the function of C1 in such a way that its interaction with C4 and C2 leads to a more efficient generation of C3 convertase.<sup>480</sup>

A compendium of inherited and acquired defects in complement function is found in Table 7-10.

## Methods of Examination

### Morphologic Examination

#### Biopsy of Lymph Nodes

Biopsy of enlarged lymph nodes often is an important diagnostic step in differentiating the various types of primary and secondary lymph node diseases as discussed in Chapter 40. In addition, however, a lymph node biopsy specimen may be useful in the assessment of immunologic function in patients suspected of having immune deficiency dis-

eases (Chapter 44). In such instances biopsy should be done approximately one week after local antigenic stimulation, for instance, by the injection of diphtheria or tetanus toxoid into skin areas drained by the node to be excised. Lymph nodes should be examined to assess the thymus dependent paracortical areas and the lymphoid follicles and germinal centers as well as the number of plasma cells in the medullary cords.<sup>515</sup> Lymph node biopsy is not necessary in the diagnosis of severe combined immune deficiency and may be detrimental since lymph nodes are hard to find and infection may ensue.<sup>513</sup>

*Lymph node puncture*<sup>521</sup> is also helpful in the differential diagnosis of various lymph node disorders and metastases of malignant tumors. In this procedure the overlying skin is cleansed and a 20-gauge needle, attached to a dry syringe, is inserted into the node, which is firmly held between the thumb and index finger. The tissue is aspirated rapidly and the needle is withdrawn. Smears are made on a dry slide and stained with Wright's stain.

#### Splenic Biopsy

The value and technique of *splenic biopsy* are discussed in Chapter 8.

**Table 7-10. Abnormalities of Complement System**

#### *Inherited abnormalities*

##### *Classic complement pathway*

- 1 C1—inhibitor deficiency—hereditary angioneurotic edema<sup>478</sup>
- 2 Deficiency of C1q associated with immune deficiency syndromes<sup>455, 456</sup>
- \*3 C1r deficiency<sup>454</sup>
- \*4 C2 deficiency<sup>449, 479</sup>
- 5 Familial C5 dysfunction with deficient opsonization<sup>467</sup>

##### *Alternate pathway*

- 1 Abnormality of alternate pathway with recurrent infection<sup>446, 476</sup>

#### *Acquired abnormalities*

- 1 Complement activation by abnormal immunoglobulins
  - (a) Monoclonal gammopathy<sup>452</sup>
  - (b) Mixed cryoglobulinemia<sup>475</sup>
  - (c) Lymphosarcoma with IgM monomers<sup>482</sup>
- 2 Hypocomplementemic chronic glomerulonephritis<sup>437, 446, 459, 493</sup>
- 3 Decreased synthesis in liver disease<sup>471</sup>

\* High incidence of autoimmune diseases

## NORMAL HUMAN SERUM

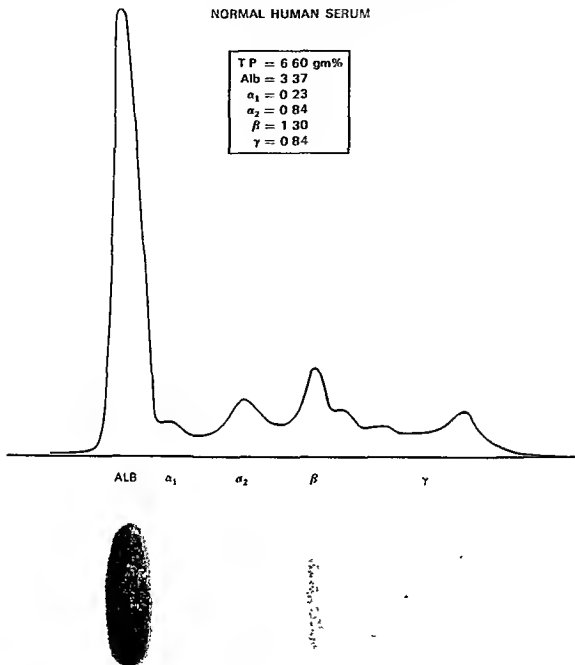


Fig 7-21 Normal electrophoretic pattern of serum proteins. Cellulose polyacetate, Gelman Sepharose III system (Courtesy of M. McMorran and F. Paraskevas, Manitoba Institute of Cell Biology)

## Rectal Biopsy

Rectal tissue is readily accessible for biopsy and may be examined for plasma cells, either by ordinary histologic procedures or

by immunofluorescence techniques. Absence of plasma cells from the lamina propria reflects a defect of the secretory immunoglobulin system.<sup>505</sup>

*Lymphangiography* is the radiologic study

of radiopaque, contrast-filled lymph nodes and vessels that cannot be examined by simple clinical means, such as those of iliac and para-aortic chains. A radiopaque dye is injected into the lymphatic channels of both feet and is carried into the above-mentioned nodes. The clinical application of this technique, its usefulness, limitations, and dangers are discussed in Chapter 40.

### Functional Assessment of Humoral Immunity

For routine clinical purposes the following procedures are recommended: (1) a preliminary assessment of serum proteins by electrophoresis or immunoelectrophoresis, (2) quantitative measurement of various immunoglobulins, eg, IgG, IgA, IgM, etc.; and (3) assessment of the capacity to produce specific antibodies.

### Immunoglobulin Concentrations

#### *Protein Electrophoresis*

Proteins migrate in an electric field except at the pH of their isoelectric point. The rate of migration depends on the net charge of the protein and hence on the pH and ionic strength of the solution, and, to a lesser extent, on the size and shape of the molecule. Electrophoresis may be carried out in solution or by using porous inert media such as starch, silica gel, or, most often, moistened filter paper. Figure 7-21 shows the electrophoretic pattern obtained with normal human serum. The peaks represent groups of proteins separated on the basis of their mobility: albumin,  $\alpha_1$ - and  $\alpha_2$ -globulin,  $\beta$ -globulin, and  $\gamma$ -globulin. Antibodies occur predominantly in the  $\gamma$ - and  $\beta$ -globulin peaks. It is possible to assess the proportion of different components by measuring the fraction of the total area attributable to each component.

#### *Immunoelectrophoresis*

A more satisfactory separation of serum proteins is obtained by *immunoelectrophoresis*, a technique that makes possible the differen-

tiation of proteins on the basis of electrophoretic and immunologic properties. Separation of individual proteins is achieved by electrophoresis in agar gel. Later, antisera reacting with one or all plasma proteins are added to a long trough in the agar gel, beside the separated protein fractions. Within a few hours precipitin lines form at points of contact between the diffused proteins and their specific antisera in the trough. With this technique a large number of serum proteins have been identified by the characteristic position, shape, and intensity of their precipitin lines (Fig. 7-22).

### Quantitative Assessment of Immunoglobulins

The concentration of specific immunoglobulins is best determined by a variety of techniques that depend on the precipitation of proteins by specific antisera. They include single radial diffusion and double diffusion in agar gel,<sup>508, 518</sup> immunoelectro-diffusion,<sup>511</sup> and radioimmunoassay.<sup>522</sup> In all these techniques the immunoglobulin concentration of the test serum is compared with a standard solution of defined concentration.

Levels of IgG subclasses may also be measured by the single radial diffusion assay.<sup>518</sup> When dealing with extremely low concentrations of immunoglobulins, inhibition of hemagglutination tests has been found useful.<sup>523</sup> Secretory immunoglobulins may be measured following concentration of secretions obtained from parotid saliva, the gastrointestinal tract, and elsewhere.

Normal immunoglobulin levels for various ages are found in Appendix A (Table A-24).

### Antibody Formation

Titers of "naturally occurring" antibodies such as A and B isohemagglutinins, heteroagglutinins (eg, against sheep red cells) and antibodies against ubiquitous bacteria or their products (eg, antistreptolysins) are useful in screening for humoral immune deficiencies. Most children have been immunized against diphtheria, tetanus, and pertussis. Determination of antibody titers against these speci-

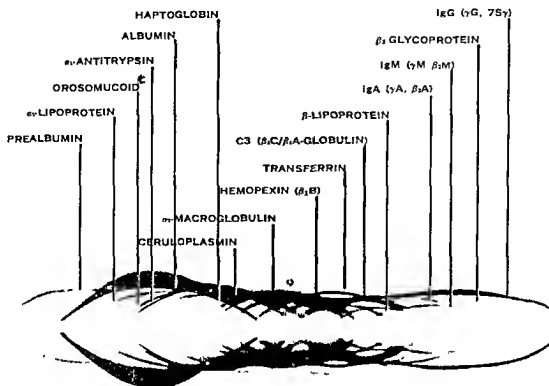


Fig 7-22 Immunoelectrophoretic pattern of normal human serum, using horse antiserum (Courtesy of Hyland Division Travenol Laboratories Inc)

ficties, preferably following a recent booster, is of great value. Other harmless antigens, which may be useful for active immunization, include the polysaccharides derived from pneumococci, *H. influenza* and *N. meningitidis* antigens,<sup>513,516</sup> the Vi antigen, and flagellin.<sup>520</sup>

Live vaccines such as vaccinia, polio, measles, rubella, BCG, and tularemia should *never* be used in patients suspected of having an immune deficiency.

Recommended vaccines, dosages, and intervals between immunizations are listed in Table 7-11.<sup>513</sup> Preferred methods for determination of antibody levels have been published.<sup>513</sup>

#### Functional Assessment of Cellular Immunity

Tests commonly employed for assessing cell-mediated immunity include (1) skin tests

for delayed hypersensitivity, (2) *in vitro* stimulation of lymphocytes to divide and form blast cells, and (3) the assessment of functions mediated by soluble lymphocyte products.

#### Skin Tests

Skin tests must be done with a battery of antigens, since universal sensitivity to any single agent does not exist. In varying dilutions, 0.1 ml of the following antigens is injected intradermally<sup>513</sup>; skin tests are read 48 hours later and the degree of erythema and induration is recorded in millimeters: (1) candida 1:10 for infants, 1:100 or 1:1000 for older children and adults; (2) tuberculin 1:10,000, if negative repeat at 1:1000; (3) Trichophyton, as for candida; (4) streptokinase-streptodornase, at a concentration of 5 units per 0.1 ml, if negative repeat at 40 units per 0.1 ml; (5) mumps antigen in stand-

ard dilution. Additional antigens include coccioidin and other agents having a high rate of reactivity in specific geographic regions.

*Active sensitization* may be carried out with contact allergens such as 2,4-dinitrochlorobenzene (DNCB).<sup>503</sup> This agent is highly irritating, especially in young children, and unpleasant burn reactions may occur. It should *never* be injected intradermally! DNCB is dissolved in acetone to form a stock solution of 2000  $\mu\text{g}$  per 0.1 ml. Sensitization is carried out with doses of 1000 to 2000  $\mu\text{g}$  applied to the volar surface of the forearm. To prevent spreading over a large area, a stainless steel or plastic ring measuring 2 cm in diameter is pressed to the volar aspect of the forearm which is held in the horizontal position. The sensitizing dose is put into the ring. After the solution dries the area is covered for 24 hours. The site is examined at 14 days for a spontaneous flare, which denotes sensitization to remaining antigen. In the absence of a spontaneous flare a challenge dose of 50 to 100  $\mu\text{g}$  per 0.1 ml is applied and the reaction is read at 48 hours. Induration denotes a positive response, erythema alone does not. Patients with unusually strong reactions may also show vesiculation and ulceration in addition to induration.

Keyhole limpet hemocyanin also produces cellular immunity, but is not recommended for general use.<sup>513</sup>

### Lymphocyte Transformation

The small lymphocyte can be activated *in vitro* and *in vivo* to alter its structure and to express latent functions.<sup>1,13,24,354</sup> The process is known as "lymphocyte transformation" and must not be confused with that occurring in virus-induced neoplasia.

*In vitro* transformation may be induced by a variety of agents,<sup>24,354</sup> some of which stimulate most cells, whereas others stimulate only a select few. The former are exemplified by phytohemagglutinin (PHA), pokeweed mitogen (PWM), concanavalin A (ConA); and antilymphocyte serum, the latter by specific antigens.

### Biochemical Events in Transformation

(1) The first step in lymphocyte activation is thought to be the interaction of mitogens with a variety of specific membrane receptors.<sup>506,507</sup> (2) In PHA-stimulated cells this step is followed almost immediately by a

**Table 7-11. Recommended Immunization Procedures for Evaluation of Antibody Production**

Vaccine	Dose	Number of Doses	Interval between Doses	Time of Antibody Determination (in weeks after last dose)
OPT	0.5 ml IM	3	1 week	2
Polio (killed)	1.0 ml IM	3	2 weeks	2
Pneumococcal polysaccharide	0.1 mg IM	3	1 week	2
H influenza polysaccharide	0.05 mg SC	1	—	2
N meningitidis polysaccharide	0.05 mg SC	1	—	2
Vi antigen (E Coli)	0.1 mg SC	1	—	2
Flagellin	5 $\mu\text{g}$ SC	1	—	2

IM, intramuscularly, SC, subcutaneously



greatly increased turnover (up to 21-fold in 30 minutes) of membrane phosphatidyl inositol,<sup>509</sup> and is followed in the next 24 hours by an increased accumulation of all phospholipids.<sup>510</sup> (3) Within the first hour there is increased acetylation of histones (the basic nucleoproteins complexed with DNA), which appears to precede the increase in nuclear RNA synthesis and may indicate changes in the fine structure of chromatin and the ability of DNA to serve as a template for RNA synthesis.<sup>519</sup> (4) Increased synthesis of RNA, predominantly polydisperse RNA, occurs within 30 minutes to two hours,<sup>504,507</sup> and is preceded or followed immediately by increased protein synthesis<sup>519</sup> which includes new ribosomal proteins. (5) Morphologic changes, enlargement of the cell and reorganization of nuclei and nucleoli (described on page 287) begin within 24 hours, followed by increased endocytosis and redistribution of acid hydrolases within lysosomes.<sup>502</sup> (6) At 24 to 36 hours there is a gradual increase in the incorporation of <sup>3</sup>H-thymidine (<sup>3</sup>HTdR)<sup>285,507</sup>; for specific antigens the onset of <sup>3</sup>HTdR incorporation is delayed for six to 12 hours.

The S-phase (DNA synthetic phase) lasts for six to 10 hours and is followed by the G<sub>2</sub> phase, a premitotic phase, which lasts for two to four hours, and is accompanied by the synthesis of RNA and protein. The cell then undergoes mitosis.

### Laboratory Studies

**LYMPHOCYTE STIMULATION.** Lymphocyte stimulation may be evaluated in terms of morphologic changes or by the incorporation of <sup>3</sup>HTdR, measured either by radioautography or by direct counting of radioactivity. For clinical purposes the last is the most convenient and useful test, but it must be noted that morphologic changes do not parallel thymidine uptake in all situations.<sup>512</sup> Lymphocyte cultures are generally set up at concentrations of 1 to 3 × 10<sup>6</sup> cells per tube and contain appropriate concentrations of stimulants. The response in stimulated cultures is then compared to that of unstimu-

lated cultures. It is always essential to determine the phytohemagglutinin response of at least two normal donors simultaneously, in order to guard against nonspecific variables. The same applies to other mitogens.

The capacity to respond to PHA, ConA, and antigen *in vitro* appears to be a function of T lymphocytes,<sup>506</sup> and under most circumstances lymphocyte transformation appears to correlate well with reactions of cellular immunity *in vivo*.<sup>285,354</sup> However, discrepancies between the proliferative response to antigen *in vitro* and the ability to develop delayed hypersensitivity reactions and produce MIF have been noted, especially in patients suffering from chronic mucocutaneous candidiasis.<sup>285</sup>

In contrast to PHA and ConA, PWM appears to stimulate B cells as well as T cells.<sup>506</sup>

**MIGRATION INHIBITION FACTOR.** The interaction of sensitized T cells with antigen releases a number of biologically active materials (page 321). Release of MIF by such cells appears to be an excellent *in vitro* correlate of cell-mediated immunity *in vivo*.<sup>297</sup> The technique preferred at present involves culturing blood lymphocytes in the presence of antigen for 72 hours and adding the cell-free, concentrated supernatant to chambers containing guinea pig macrophages<sup>297</sup> (see Fig. 7-17).

**MIXED LEUKOCYTE CULTURES.**<sup>500,501</sup> When lymphocytes from unrelated individuals are mixed and allowed to grow *in vitro*, transformation occurs and apparently reflects a primary immune response *in vitro*. In this system lymphocytes function not only as mediators of immunity but also as carriers of antigens. Standard techniques for mixed leukocyte cultures have been published.<sup>500,501</sup> In most instances one cell population is rendered incapable of proliferation and serves as antigen whereas the other population furnishes the proliferating cells (one-way test).<sup>500</sup>

Apparently the capacity to respond to allogeneic cells is a property of T cells.<sup>506</sup> In several patients suffering from immune deficiency syndromes responses were seen in this

test, although the response to PHA was impaired.<sup>506</sup>

Tests of lymphocyte cytotoxicity against allogeneic cells or syngeneic tumor cells have been described previously (page 325).

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## Methods of Examination



## *The Reticuloendothelial (Mononuclear Phagocyte) System and the Spleen*

### **The Reticuloendothelial (Mononuclear Phagocyte) System (RES, MPS)**

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#### **Definition**

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## **The Reticuloendothelial (Mononuclear Phagocyte) System (RES, MPS)**

### **History**

The term "reticuloendothelial system" was first used by Aschoff<sup>1</sup> to designate those cells, widely scattered throughout the body, that

take up acid vital dyes with great avidity. It had been shown previously that the free and fixed large mononuclear cells of Metchnikoff ("macrophages" as opposed to the blood leukocytes or "microphages") in the omentum, liver, spleen, and other tissues had great affinity for these dyes. Later it was demonstrated that the electronegative charge of the dye, the degree of dispersion of dye colloid, and the blood supply to the tissues greatly influenced the intensity of tissue staining.<sup>8,12</sup> Subsequently, India ink, iron, and other metal particles were injected into animals and the patterns of tissue uptake were recorded. It became apparent that, under appropriate conditions of injection and especially after local tissue damage, dye or particle uptake occurred in a wide variety of tissues including reticular cells of the spleen, bone marrow and liver, renal tubular cells, capillary endothelium of the adrenal cortex and hypophysis, branched cells in the thyroid and parathyroid glands, myocytes in the heart, and glial cells in the central nervous system.<sup>8</sup> Furthermore, after repeated injections of dye or particulate material an even greater variety of cells participated in such storage. For example, particles of carmine or trypan blue dye first appeared in the epithelium of the convoluted tubules in the kidney. As injections continued, particles were found in adrenal cortex and hepatic cells (both Kupffer cells and hepatocytes) which swelled and became

stuffed with dye, and in some instances (eg, liver) were transformed into giant cells. The number of storing cells also increased as a result of local cell division and recruitment of new cells. After many injections, fine dye particles appeared even in fibrocytes and in the general vascular endothelium. In short, most body cells may ultimately become phagocytic under appropriate circumstances.

Aschoff divided the various cells that ingested injected dyes into four groups: Groups I and II consisted of endothelial cells of the blood and lymphatic vessels and the fibroblasts, all of which exhibited relatively weak staining; these cells were not included in the designation "reticuloendothelial system." Group III consisted of the reticulum cells of the spleen and lymph nodes. Group IV included the cells lining the sinusoids of the liver, bone marrow and lymph sinuses, similar cells in the adrenal and pituitary glands, and the free histiocytes in the splenic sinuses and other organs. Groups III and IV were thought to constitute a single functional cell system. It was because these cells seemed to be involved in reticulum formation or lined blood or lymph sinuses that the term "reticuloendothelial system" (RES) was coined. This functional unit, however, is not a distinct, cytologically identifiable one. A number of the cells of the RES may be identified in tissue sections by their staining reactions with metal salts ("metallophil" cells), but other undifferentiated, primitive reticular cells cannot be recognized in this way.<sup>12</sup>

### Definition of the RES

Because attempts to define the "RES" have not provided an improved definition<sup>6</sup> since Aschoff's studies<sup>13</sup> we will use the following working definition: the RES consists of the reticulum cells of the spleen and lymph nodes, the cells lining the sinusoids of the liver, bone marrow and lymph sinuses, as well as similar cells in the adrenal and pituitary glands and the free histiocytes in the spleen and other organs that avidly ingest injected dyes. As discussed in Chapter 6, because these cell types are now considered to

be derived from the blood monocytes, which in turn are the products of precursors in the bone marrow, they are increasingly being referred to as cells of the mononuclear phagocyte system (MPS) rather than of the RES. However, since the older literature refers to the RES it will often be necessary to use that designation.

### Size of the RES or MPS

Because the component cells are so diffusely distributed, the size of the RES is unknown. Nevertheless, estimates of the RE cell component of three of the major RE organs (the spleen, liver, and bone marrow) in the rat yielded approximately equal values for each of the organs, ie, about one billion RE cells.<sup>9,10</sup> From similar studies of organ weight and DNA content, the normal human spleen was estimated to contain 140 billion cells.<sup>9</sup> If it is assumed that about half of the total cells present in the spleen are RE cells (as in the rat) and that the liver and bone marrow contain an equal number of RE cells, the RES of man consists of at least 200 billion cells and constitutes a diffusely distributed organ of considerable size.

### Functions of the RES or MPS

In the spleen and lymph nodes the cells of the RES lie in close structural proximity to immunologically reactive cells, whereas in the liver and bone marrow these cells are in close contact with hepatocytes and hematopoietic cells, respectively. These associations suggest somewhat different and perhaps specialized functions of the RE cells according to their location. Nevertheless, the common denominator of the system as a whole appears to be the engulfing of particulate material and effete or damaged cells. The end result of such ingestion may vary from rapid killing and digestion of bacteria or red cells to indefinite storage of particles such as silica, carbon, and thorium dioxide. The clearing function of the system is thus fundamentally related to body defense.

### Clearing Capacity

The clearing capacity of the RES or MPS was quantitated by injecting increasing doses of carbon particles, saccharated iron oxide, colloidal gold or silver, thorium dioxide, or aggregates of serum protein into animals.<sup>3,4</sup> It was demonstrated that: (1) with increasing doses, the rate of particle clearance asymptotically approaches a maximal clearance rate; (2) in a given species the maximal clearance rates of different colloids are not the same; and (3) the maximal clearance (expressed as mg/100 g body weight) for a given colloid (eg, carbon) differs from species to species.<sup>3,4</sup> This variation in clearing capacity appeared to be a function of the weight of the spleen and liver since the clearance per 100 g of liver plus spleen was approximately the same in the several species studied.<sup>3</sup> It was also noted that maximal clearing capacity decreased as the animals grew older.

In rodents the clearance of injected colloids from the blood was best described by a single exponential function. At doses lower than the maximal clearance capacity of the system, about 85% of the colloid was found in the liver and 5% in the spleen. As the dose of injected colloid was increased, clearance by the spleen increased to about 20% of the injected dose.<sup>2</sup> The predominant clearance by the liver was thought to reflect differences in blood flow to the two organs and, in fact, the clearance rate of small doses of dye or other colloids is used to measure liver blood flow.<sup>2</sup> Since metal and dye particles are not metabolized and remain in the RES almost indefinitely, study of the RES clearing function in man became possible only when it was demonstrated<sup>7</sup> that, after measuring the clearance rate of aggregated serum albumin at several intermediate doses, the maximal clearing capacity could be calculated by using the analytic method of Michaelis-Menten.<sup>7</sup> The maximal rate of phagocytosis of aggregated albumin was found to be 1.07 mg/kg/min in man.<sup>7</sup>

The site of particle clearance by the RES is influenced by blood flow, local tissue damage, presence of opsonins, the nature of the

particles, and probably other factors. Thus, in man, the spleen has a special ability to clear mildly damaged erythrocytes from the circulation, whereas more severely damaged red cells are removed mainly by the liver.<sup>11</sup> Other sites are stimulated to activity under other conditions. For example, the RE activity of the bone marrow was found to be increased after the induction of marrow hypoplasia in rats.<sup>10</sup>

### Blockade of the RES

The clearing function of the RES can be blocked to a variable extent by the injection of particle suspensions (eg, carbon, saccharated iron oxide, thorotrast). That RES blockade is fairly specific was shown by the observation that clearance of a given particle is decreased more by prior injection of a blocking dose of that same particle than by injection of a different one.<sup>14</sup> The mechanism of this blockade specificity is unknown but the blockade may reflect saturation of a specific population of phagocytic cells or depletion of serum factors (opsonins) needed to facilitate phagocytosis. Morphologic examination after the simultaneous injection of a dye and a metal colloid demonstrated that some RE cells contained only dye while others contained only metal particles, thus supporting the concept that there are populations of phagocytic cells with a special affinity for different types of particles.<sup>18</sup> However, still other studies using chronic phosphate in rats suggest that RES blockade is not the result of general depression of RE cell function, saturation of particle specific cell clones, or depletion of serum opsonins. Rather, it was postulated that RES blockade occurs when large numbers of particles saturate the binding sites of macrophage membranes.<sup>5</sup>

The duration of RES blockade by nontoxic particulate matter such as carbon particles was found to be transient (less than 24 to 48 hours in rats) and recovery was associated with enlargement of the liver and spleen.<sup>3</sup> From this it was concluded that new cell formation, either locally or from recruited blood monocytes, was a major factor in re-

covery from blockade,<sup>3</sup> and it was demonstrated that cortisone or nitrogen mustard delayed recovery, presumably by depressing cell proliferation.<sup>4</sup> The duration of blockade varied with the dose given and with the type of particle; also blockade produced by readily metabolizable particles, such as aggregated albumin, lasted only three to four hours while the effects of gelatin and colloidal gold were more prolonged.<sup>18</sup> Nevertheless, recovery after blockade with nonmetabolizable particles does occur. Such materials become enclosed in intracellular vacuoles.<sup>19</sup>

### *Function of the RES in Body Defense*

Transient depression of RES phagocytic activity followed the intravenous injection of endotoxin or killed *Salmonella typhosa*.<sup>15,20</sup> This was succeeded by an increase in the weight of the liver and spleen and thereafter the phagocytic capacity was found to return to and exceed the normal range, just as after carbon injection.<sup>4</sup> However, the injection of virulent organisms into mice (*S. Danysz*, *S. typhimurium*, and several strains of tubercle bacilli) produced similar changes initially, but then damage to the RES supervened, small abscesses appeared, and most of the mice died.<sup>4</sup> The size of the infective dose and the virulence of the organisms appeared to be the main determinants of the outcome. Not all bacteria alter RES (MPS) activity since dead staphylococci when injected had no effect on the system.<sup>4</sup> Significantly, artificial stimulation of the system by serial injections of aggregated serum proteins markedly enhanced the ability of rats to resist experimental infection with *S. typhimurium* (95% survival after 17 days in the treated group as compared to almost 100% mortality in the controls),<sup>4</sup> and RES blockade increased the lethality of certain induced infections<sup>4</sup> or rendered naturally resistant animal strains susceptible. Pneumococcal pneumonia, typhoid fever, or tularemia in man enhanced the phagocytic capacity of the RES as measured by aggregated albumin clearance.<sup>16</sup> The persistence of increased RES function for several days after cessation of bacteremia was interpreted as indicating a nonspecific, de-

bris-clearing function.<sup>16</sup> In contrast, the viral infections, sandfly fever, and dengue decreased RES function as measured by aggregated albumin clearance,<sup>16,17</sup> possibly by interfering with energy sources in the phagocytes.<sup>4</sup>

The inability to kill certain organisms may explain some chronic diseases such as tuberculosis, syphilis, and chronic granulomatous disease, and inability to completely metabolize a variety of cellular components appears to explain Gaucher's disease and other storage disorders (see Chapter 42).

## **The Spleen**

The spleen intrigued Galen in the third century and many physicians and investigators since then,<sup>113,114</sup> but only in very recent times has it begun to be less of an organ of mystery, the "mysterium plenum organum" of Galen. That the spleen is closely related to the hematopoietic system is evident since it becomes enlarged in association with a variety of blood diseases and some of these diseases are ameliorated when it is removed. Furthermore, striking changes are found in the blood after splenectomy. However, what functional role the spleen normally plays and how these functions are carried out have only recently begun to be understood. Before discussing these, the unique structure of this organ needs to be understood.

### **Structure<sup>29,31</sup>**

In adult man the spleen is enclosed in a connective tissue capsule that is several millimeters thick. The capsule contains few muscle cells, and thus the human spleen is not capable of the marked contractions seen in the spleen of the cat or dog. From the internal surface of the capsule an extensive, branching network of trabeculae divides the organ into communicating compartments. This network is well seen in spleens digested to show the gross trabecular structure.<sup>31</sup> The appearance is not unlike that of a sponge, with the spaces containing the parenchymal or pulp tissue. The parenchyma is composed

of two types of tissue, the *white pulp* and the *red pulp*. These will be described below.

The splenic capsule is deeply indented on the medial surface where the nerves, lymphatics, and blood vessels enter. The lymphatics are restricted to the capsule and *trabeculae* and are essentially absent from the parenchyma. The nerves are those of the sympathetic nervous system and are associated with the blood vessels well out into the pulp, perhaps as far as the terminal capillaries. The splenic artery branches at the hilum into multiple *trabecular arteries* that in turn give off branches that leave the trabeculae

and enter the parenchyma (Fig. 8-1). The branches of the trabecular arteries, called *central arteries* because of their central position in a sheath of white pulp, are medium-sized, muscular vessels that can markedly vary blood flow by their contraction or relaxation.<sup>26</sup> From them and their secondary branches even smaller branches are given off in a radial fashion and these enter the white pulp where, after further branching and arborization, they terminate in the marginal sinus or enter the marginal zone of the red pulp (Fig. 8-2).<sup>30</sup> After distributing multiple branches to or through the white pulp the

### WHITE PULP

### RED PULP

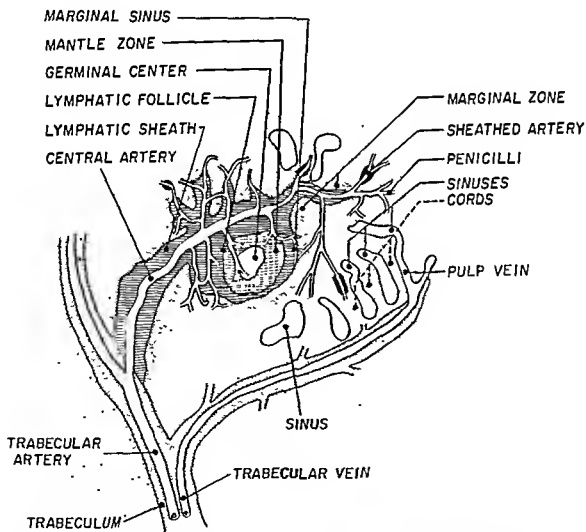


Fig. 8-1. Diagram of the splenic structure and circulation. The components of the white pulp are listed on the left, those of the red pulp on the right.

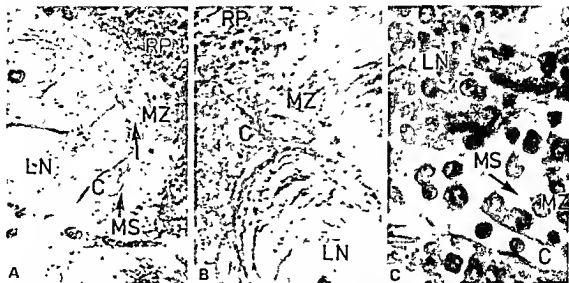


Fig. 8-2 A Capillaries (C) in the rat spleen are shown traversing a lymphatic node (LN) of the white pulp and entering the marginal sinus (MS). The marginal zone (MZ) and the red pulp (RP) are seen at the right (X 180). B A white pulp capillary (C) is shown leaving the area of the lymphatic node (LN) and traversing the marginal zone (MZ) to enter the red pulp (RP) (X 190). C A capillary (C) of red pulp origin opens into the marginal sinus (MS). The arrow indicates a marginal sinus pore (X 740). (From Snook,<sup>28</sup> courtesy of the author and Wistar Institute of Anatomy and Biology.)

attenuated main central artery divides into several branches that run on into the red pulp. These branches are the *penicilli* (vessels about 25  $\mu$ m in diameter which contain in their walls smooth muscle, basement membrane, and adventitial cells [Fig. 8-1]). They may terminate or continue on to become arterial capillaries, some of which may be sheathed by closely packed phagocytic cells and reticular fibers.<sup>23</sup> Most of these capillaries appear to terminate in the cordal tissue of the red pulp or in the marginal zone. Only a rare vessel terminates in close proximity to or directly connects with a sinus.<sup>25,31</sup>

The supporting structure of the parenchyma consists of a diffuse network of large, branched or stellate reticular cells and the reticular fibers that they produce. On electron microscopy these fibers can be seen within, as well as outside, the reticular cells, and in the latter location they are often partially ensheathed by reticular cell cytoplasm. The reticulum is morphologically and immunologically related to basement membrane and cement substance.<sup>31</sup> The arrangement of reticular cells and fibers is somewhat different in

the white pulp than in the red pulp and the cells interspersed within the pulps differ also.

In the *white pulp* the reticular cells and fibers are circumferentially and loosely arranged around the central artery. In this meshwork, predominantly small lymphocytes plus variable numbers of plasma cells, macrophages, and a few other cells lie free (*lymphatic sheaths*); red cells are few in number. Adjacent lymph follicles, eccentrically located in relation to the central artery, comprise a major portion of the white pulp (Fig. 8-1).

The *red pulp* consists almost entirely of thin-walled *splenic sinuses* and the *splenic cords* which lie between them. The sinuses are vascular channels incompletely lined with elongated, tapered cells arranged with their long axes parallel to that of the vessel, rather like the staves of a barrel. There is some disagreement about whether the cells lining the sinuses are modified reticular cells or endothelial cells,<sup>20,21,25,28,31</sup> but in any case their cell borders abut one another at their lateral surfaces, and there are no attachments or desmosomes, nor is there intercellular ce-

ment substance between them. As a result the lining cells may be easily separated, and small gaps between them are often seen through which blood cells traverse the sinus wall or pseudopods of macrophages extend (Figs. 8-5 and 8-6). A heavily fenestrated network of reticular, basement membrane fibers surrounds the sinus, like a roll of chicken wire, and these fibers anastomose with the reticular fibers of the cordal tissue. As already mentioned, the *splenic cords* lie between the sinuses. They consist of blind sacs, with no morphologically intact openings into the sinuses. The cords are filled with a meshwork of reticular cells together with extracellular reticulum quite similar to that of the white pulp with which they merge almost imperceptibly in the marginal zone. Between the reticular cells of the cords may be found a diverse cell population. Lymphocytes and monocytes are present in high concentration as are macrophages and erythrocytes; neutrophils and platelets are considerably less abundant.

### Blood Flow through the Spleen

Whether blood flow through the spleen follows anatomically discrete vascular channels ("closed" circulation) or empties into pulp areas and somehow traverses them to reach the sinuses via an "open" circulation has been the subject of controversy for years. Knisely, using quartz rod transillumination and microscopic observation of exteriorized spleens, saw no vessels opening out into or pouring blood into intercellular pulp spaces in living unstimulated spleens.<sup>23,24</sup> He described blood flow through a vascular system consisting of a series of completely interconnected, preformed channels as well as cyclic filling and emptying of the splenic sinuses, apparently regulated by afferent and efferent sphincters. For a time his studies appeared to settle the controversy in favor of the "closed" circulation. However, other workers using the same techniques<sup>25</sup> were unable to confirm Knisely's findings. This fact plus the inability of many workers<sup>20,21,25,29,30,31</sup> to find morphologically discrete channels on

careful light and electron microscopic study has resulted in rejection of the "closed" circulation theory and there is now general acceptance of the "open" circulation hypothesis. According to this, arterial blood is normally delivered to the marginal sinus, the marginal zone, and the red pulp (Fig. 8-2) and from here the blood rapidly traverses the intervascular zone. In the larger, most direct pulp channels, the rate of flow may be scarcely less than in the arterial capillaries. In the smaller, more devious passages, progress is slower and irregularly intermittent.<sup>26</sup> Thus, normally most of the blood appears to reach the sinuses by direct but anatomically undefined channels, and only a small proportion (<2%) slowly percolates through the labyrinthine cordal tissue. From the sinuses the blood flows directly into pulp veins and thence into trabecular veins. The blood supply to the white pulp comes from the radial branches of the central artery. The paucity of erythrocytes in the white pulp apparently results from plasma skimming at the right-angle branching of the radial arteries. These arteries divide into capillaries that arborize and finally terminate in the marginal sinus (Fig. 8-2).

### Erythrocyte Mixing in the Spleen

In man, radioactivity measurements over the normal spleen after the injection of <sup>51</sup>Cr-tagged, normal erythrocytes demonstrated a rapid, single exponential increase to equilibrium levels in from 15 seconds to 2 minutes<sup>103,120</sup> (Fig. 8-3). This and the fact that splenic mixing curves after injections of labeled red cells and labeled plasma were essentially identical<sup>27</sup> clearly demonstrated the lack of significant trapping of normal red cells in the normal human spleen.

In contrast, in animals, such as the cat, which have many muscle cells in the capsule and trabeculae, variation in blood content of the pulp is controlled by relaxation and contraction of the whole organ.<sup>28</sup> In such animals a double exponential, splenic mixing curve was seen after the injection of <sup>51</sup>Cr-labeled erythrocytes (Fig. 8-3) thus demonstrating a



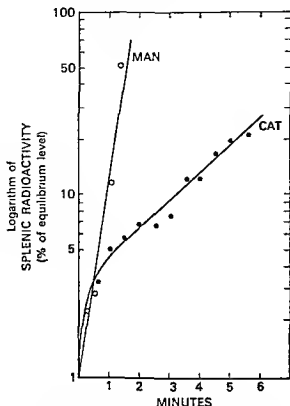


Fig 8-3 Splenic red cell-mixing curves obtained after the injection of radioactively labeled, autologous erythrocytes into man (o) or the cat (•). Note the single exponential mixing curve in man and the two component curves in the cat (Idealized curves adapted from Pranker<sup>21</sup>)

considerable amount of splenic red cell pooling even in the normal state.<sup>27</sup> This splenic pool of red cells could be mobilized by the injection of  $\alpha$ -epinephrine.<sup>27</sup> A variety of stresses such as excitement, anoxia, hemorrhage, exercise, and electric stimulation or manipulation of the splenic pedicle also result in splenic contraction. In animals, splenic relaxation occurs after eating, in response to certain anesthetic agents, and to some degree in response to autonomic blocking agents.<sup>26,27</sup> Intermittent arterial constriction additionally regulates blood flow to areas of the pulp.<sup>26</sup> In man, however, autonomic blockade did not result in red cell pooling in the spleen,<sup>27</sup> and alteration in arterial vessel size is presumed to be the major mechanism for variations in splenic blood flow.<sup>23</sup>

### Platelet Mixing in the Spleen<sup>22</sup>

In normal man, after the injection of  $^{51}\text{Cr}$ -labeled autologous platelets, the splenic mixing curve, like that for red cells (Fig. 8-3), followed a simple exponential pattern.<sup>22</sup> However, the half time for platelet mixing in the spleen was 2.5 minutes and mixing was complete only after about 10 minutes. Another difference between erythrocytes and platelets is that, although there appears to be little or no splenic reservoir of erythrocytes in man, approximately 30% of the normal circulating mass of platelets resides in the spleen. These platelets can be expressed into the circulation during a slow infusion of epinephrine.<sup>22</sup>

### Changes in the Blood Following Splenectomy<sup>27,41,60,66</sup>

Since some of the functions of the spleen have been inferred from the changes in the blood which can be observed following splenectomy or are found when the spleen is congenitally absent, these will be considered first.

In man and in animals,<sup>50</sup> splenectomy results in changes in the morphologic structure of red cells and in the concentration of platelets and leukocytes. Similar changes occur both after the removal of the normal spleen (eg, trauma) and after removal of the spleen for treatment of pathologic states.

The most striking changes are seen in the red cells (Fig. 8-4). Nucleated red cells may appear in the circulating blood as well as corpuscles containing Howell-Jolly bodies; the percentage of reticulocytes may be increased<sup>41</sup> and siderocytes, diffuse basophilia, and target cells are found.<sup>41,52,60</sup> Autophagic vacuoles, which appear to develop even in normal erythrocytes, also accumulate in the blood in increased numbers.<sup>48</sup> These changes may be more striking when red cell production is stimulated or otherwise altered, as can be seen in splenectomized dogs or pigs in which anemia was produced by phlebotomy, the administration of acetylphenylhydrazine, or by pyridoxine deficiency.<sup>38,39</sup> For exam-

ple, in the splenectomized dog made anemic by bleeding or injections of acetylphenylhydrazine, the number of normoblasts in the blood was four times greater than in non-splenectomized animals.<sup>38</sup>

Howell-Jolly bodies are the most consistent finding in the blood following splenectomy. They are seen in all such patients and persist for years.<sup>60</sup> The erythrocyte volume remains normal after splenectomy, but the erythrocyte surface area is larger<sup>60,62</sup>; the corpuscles are thinner (leptocytes) and may take the form of target cells (see Chapter 3). The saline osmotic fragility of these cells is decreased<sup>60</sup>; however, when examined many years following splenectomy this often,<sup>41</sup> but not always,<sup>60</sup> has returned to normal. There appears to be no consistent change in erythrocyte or hemoglobin concentration after splenectomy,<sup>52</sup> both polycythemia (6 to

$7.5 \times 10^{12}$  cells/l) and anemia having been reported.<sup>41</sup> The latter is mild and usually is transient. Red cell life span is not detectably altered.

After splenectomy, *leukocytosis* occurs; it is usually modest ( $10.0$  to  $15.0 \times 10^9$  cells/l) and may occasionally be quite pronounced ( $30.0 \times 10^9$  cells/l). The maximum increase is reached during the first postoperative week, and is due chiefly to an increase of neutrophils. Patients examined three months or more after removal of the spleen showed slight to moderate leukocytosis, but this was due mainly to an increase in lymphocytes and monocytes and not to neutrophilia<sup>56</sup>; the same findings have been reported for rats.<sup>63</sup> Less consistently, eosinophilia and basophilia are found.<sup>52</sup> The postsplenectomy changes may persist for years.

*Thrombocytosis* occurs after splenectomy in

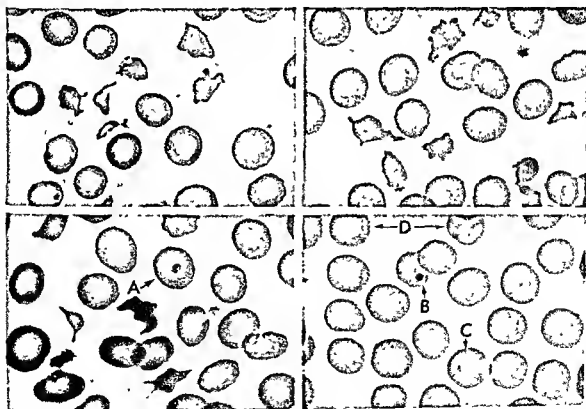


Fig 8-4 Photomicrographs of blood smears of patients splenectomized because of idiopathic thrombocytopenic purpura. Note the bizarre poikilocytes, the target cell (A), the Howell-Jolly body (B), the stippling (C), and the barely perceptible Pappenheimer bodies (D) (Wright's stain X 1270).

spleen,<sup>86</sup> differentiate into erythroid, myeloid, or megakaryocytic precursors, and protect the recipient from the fatal effects of the irradiation.<sup>107</sup> Presumably this hematopoietic potential is also present in man, is retained throughout life, and probably explains the *myeloid metaplasia* occasionally seen in patients with severe anemia, or with damaged or infiltrated marrows.

In contrast to the lack of neutrophil, erythrocyte, and probably platelet production in the normal human spleen, in normal rats extensive uptake of injected <sup>3</sup>HTdR by splenic mononuclear cells was demonstrated,<sup>97</sup> thus indicating new cell formation in these cell lines. In these studies, two labeling patterns were seen: (1) heavy grain counts in 2 to 4% of the medium-sized to large mononuclear cells in the red pulp and in the perfollicular regions (marginal and mantle zones) and (2) low grain counts (one half to one third of those seen in [1]) in 40 to 50% of germinal center cells in the lymphatic follicles. The perfollicular and red pulp labeling appears to reflect the formation of macrophages, lymphocytes, and plasma cells and is markedly increased after the injection of heat-damaged red cells<sup>9</sup> or antigenic material.<sup>74</sup> The cells in the germinal centers which showed extensive but low grain-count labeling may represent those which provide so-called "immunologic memory." In these sites, newly formed cells appear to die early, as judged by studies which revealed labeled pyknotic nuclei ("tingible bodies") in the germinal centers in less than one-half hour after <sup>3</sup>HTdR injection.<sup>97</sup> Furthermore, the observation that germinal centers develop in lymph nodes only on secondary immunologic stimulation<sup>90</sup> provides support for the concept that a mechanism for immunologic memory operates in the germinal centers.

In any case there are at least two different patterns of new mononuclear cell production in the normal spleen: (1) that in the perfollicular regions and red pulp and (2) that in the germinal centers of lymphoid nodules. This new cell formation is greatly accentuated in spleens stimulated to increase filtering activity, as by the injection of bacterial endo-

toxin,<sup>89,83</sup> methyl cellulose,<sup>104,124</sup> saccharated iron oxide,<sup>11</sup> polyvinylpyrrolidone,<sup>82</sup> zymosan,<sup>100</sup> phenylhydrazine, and heat-damaged or stored red cells.<sup>9</sup> Repeated injection of such materials into animals results in splenomegaly, and eventually a sustained increase in mononuclear cell proliferation occurs which resembles neoplasia.<sup>9</sup> The extent of increased cell proliferation is indicated by the tenfold increase in spleen weight that follows three to four months of phenylhydrazine administration and the 18- to 20-fold increase in DNA levels.<sup>9</sup> The facts that stored or heat-damaged autologous cells have the same effect<sup>9</sup> and that some patients with biochemical defects in their red cells (eg, hereditary spherocytosis, pyruvate kinase deficiency, certain hemoglobinopathies) may show evidence of increased cell proliferation suggest that the stimulus to cell proliferation is phagocytosis and increased filtration rather than immunologic. These findings led to the proposal that the total particulate load provides homeostatic regulation of the RES.<sup>108</sup>

### *Phagocytosis and Related Functions (Pitting and Culling)*

Electron microscopic examinations of the phagocytosis of carbon particles<sup>87</sup> have added considerably to our understanding of the process already described. Within a few seconds after injection the carbon particles were found in small clumps chiefly in the marginal sinuses, marginal zone, and adjacent red pulp. Phagocytosis by macrophages that extend cytoplasmic projections through the fenestrated basement membrane, between sinus endothelial cells, and into the sinuses could be seen during the first minute (Fig. 8-5). Phagocytosis by macrophages within the cords was also evident at this stage, and platelet aggregation around the particles and their ingestion were evident both in the sinuses and in the cords.<sup>87</sup> Carbon was rarely seen in neutrophils. Carbon was first seen in sinus endothelial cells after six minutes, and by one hour heavy concentrations became evident in individual macrophages, especially those immediately beneath the basement mem-

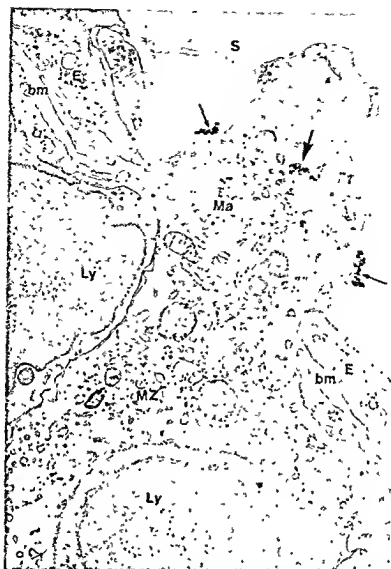


Fig 8-5 Electron microscopy of the marginal zone (MZ) of rabbit spleen 20 seconds after the injection of carbon particles. A macrophage (Ma) has projected a large pseudopod through gaps in the basement membrane (bm) and between endothelial cells (E). The carbon particles (small arrows) were found at the cell membrane adjacent to a marginal sinus (S). Phagocytosis of some particles (large arrow) was evident. Ly-lymphocyte. (From Simon and Burke,<sup>131</sup> courtesy of the authors and Harper & Row.)

brane. This concentration in splenic cord macrophages increased for at least a week and there was an associated decrease of carbon content in other organs during this interval. It was concluded that virtually all splenic phagocytosis was due to macrophages and that endothelial cells play a very minor role.<sup>67</sup> Interestingly, platelets seemed to play an important role in the clearance of carbon particles from the circulation, and this presumably

explains the reported decrease in platelet concentration after carbon ingestion.<sup>65</sup> The carbon aggregates accumulated within membrane-bound vacuoles in the macrophage cytoplasm.<sup>67</sup>

For many years the spleen has been regarded as the "graveyard" for effete and senescent blood cells<sup>132</sup> and this has been clearly established for red cells damaged experimentally and in a number of pathologic

states.<sup>131</sup> The clearance of erythrocytes severely damaged by complete or complement-fixing antibodies,<sup>83,109,111</sup> large doses of oxidant drugs,<sup>119</sup> sulfhydryl inhibitors,<sup>79,107</sup> heating,<sup>144</sup> or metal ions<sup>110</sup> appears to occur throughout the RES in a manner similar to the clearance of colloids. However, the spleen has been shown to have a special capability of trapping mildly damaged erythrocytes such as those treated with small amounts of antibody,<sup>109</sup> brief heating,<sup>144</sup> low doses of oxidant drugs,<sup>79,105,119</sup> or incomplete antibodies.<sup>109,111</sup> This unique ability of the spleen to detect minimal red cell damage or defects is presumed to depend upon its distinctive structure and vasculature in that cells traversing the tortuous cordal route are exposed to periods of stasis and come into intimate contact with splenic macrophages. Prolonged intrasplenic stasis in the cords exposes the erythrocytes to bemoconcentration, hypoglycemia, and low pH<sup>121,128</sup> and, as a result, spherocytosis and osmotic fragility are enhanced.<sup>79,119,120</sup> In mildly damaged or minimally defective cells this presumably leads to further damage of the cell membrane and, if the damage is of sufficient degree, phagocytosis follows. This "culling" process<sup>92</sup> occurs in the center of the cord.<sup>25</sup> Electron microscopic studies in normal rabbits demonstrated phagocytosis of intact whole erythrocytes, leukocytes, and platelets by macrophages in the peripheral white pulp and in the marginal zone of the splenic red pulp.<sup>138</sup> No morphologic change or fragmentation of these cells was seen outside the macrophages and after ingestion they underwent degradation in membrane-bound vacuoles. It seems possible that this process reflects normal disposal of effete blood cells in the spleen. This view is supported by studies in which a cohort of red cells was labeled by injecting <sup>59</sup>Fe into dogs and subsequently administering an excess of non-radioactive iron to obviate reutilization of the <sup>59</sup>Fe. When the radioactive erythrocytes were finally destroyed, much of the radioactivity was found in the spleen.<sup>96</sup> It has been suggested that the destruction of senescent blood cells is responsible for the maintenance of

spleen size in the normal state since splenic atrophy occurred in animals subjected to chronic phlebotomy.<sup>93</sup>

In contrast to the phagocytosis and destruction of entire cells ("culling" function), a "pitting" function of the spleen has been described.<sup>91</sup> "Pitting" refers to the ability of the normal spleen to remove particles from the intact red cell without destroying it. It was demonstrated that when blood containing a high proportion of siderocytes was transfused into recipients with intact spleens the siderotic granules were removed within several hours, but the cells, identified by <sup>51</sup>Cr labeling or immunologically, remained in the blood.<sup>91</sup> It has not yet been clearly shown that the siderotic granules are removed by "pitting," but by analogy with the removal of Heinz bodies (see below) this seems likely. The possibility remains that siderotic granule iron is also dispersed within the cell and eliminated by a metabolic process since this has been demonstrated to occur when post-phlebotomy, reticulated siderocytes are incubated *in vitro*.<sup>94</sup> On the other hand, reticulocytes induced by B<sub>6</sub> deficiency do not release their iron on incubation *in vitro* and therefore must be "pitted."<sup>102</sup>

Striking splenic sequestration of Heinz body-containing red cells (produced by phenylhydrazine injection into rabbits) and thalassemic red cells containing precipitated alpha chains of globin has been demonstrated<sup>130,139,143a,145</sup> and phagocytosis of many whole cells ensued. However, it was also shown that, as some of these damaged cells moved from the splenic cords into the sinuses, projections of cell cytoplasm, often containing the Heinz body or precipitated globin, lagged behind and were pinched off and phagocytized (Fig. 8-6).<sup>130,145</sup> This is the presumed mechanism of the "pitting" process that is thought to be unique to the spleen.<sup>25,139,145</sup> It is presumed that the same mechanism is responsible for removal of Howell-Jolly bodies, red cell nuclei, malarial parasites, bartonella organisms<sup>25</sup> and autophagic vacuoles,<sup>48</sup> but this has been less clearly documented.

A similar process appears to modify im-



Fig. 8-6. Electron microscopic view of the removal of phenylhydrazine-induced Heinz bodies in the dog spleen. The sinus lumen (SL) is above and the cordal lumen (CL) below with dark-red corpuscles seen in each. The fenestrated basement membrane can be seen with sinus endothelial cells (SE) lining the sinus lumen. A reticular cell (RC) and a phagocytic cell (Ph) are interposed between the cordal lumen and the basement membrane (BM) and the sinus lumen. Red corpuscles can be seen squeezing through the pores in the basement membrane and Heinz bodies (H) trailing behind and some of these have been pinched off and phagocytized (From Koyama et al.,<sup>25</sup> courtesy of the authors and *Mie Medical Journal*).

mature red cells<sup>101</sup> and stress reticulocytes.<sup>89</sup> Reticulocytes exhibit some of the properties manifested in erythrocytes coated with antibodies, namely, increased agglutinability (apparently related to coating with transferrin), and these cells are selectively trapped in the spleen.<sup>108</sup> Studies in which stress reticulocytes were labeled with glycine-<sup>14</sup>C (in heme) and radiophosphate (in membrane phospholipids) and then transfused into normal recipients demonstrated selective removal of labeled lipid with continued survival of the heme-labeled red cells.<sup>89</sup>

No similar "pitting" or "culling" functions

have yet been demonstrated for cells other than erythrocytes.

### Iron Metabolism

After sequestration and destruction of damaged or effete red cells, the spleen appears to be able to readily recycle the iron to the marrow to be used again for hemoglobin synthesis.<sup>25,92</sup> After splenectomy, serum iron values tend to be low for a considerable period of time, possibly because of loss of the spleen's iron-recycling function. Other organs that are sometimes involved in

hemoglobin breakdown, such as the epithelial cells of the renal tubules and the phagocytes of the lungs, do not seem to be capable of returning the iron to available body stores. When hemosiderin is deposited in the renal tubules, hemosiderinuria occurs and the iron is lost as the tubule cells are sloughed off in the urine. When bleeding occurs in the lungs, pulmonary siderosis develops and the iron is retained in macrophages, even in the face of iron deficiency anemia.

### *Antibody-Producing Function*

The importance of the spleen in antibody production has been appreciated for some years in that splenectomy was demonstrated to reduce the ability of animals<sup>75,133,135,146</sup> and man<sup>134</sup> to produce antibodies and to respond to small doses of soluble antigen. In more recent direct studies, spleen cells have been shown to produce antibody after the injection of, for example, sheep red cells<sup>74,81</sup> or poliovirus.<sup>141</sup> Autoradiographic study has shown that radioactively labeled antigens injected intravenously are rapidly taken up by phagocytes of the RES. After a single intravenous injection of bovine serum albumin or keyhole limpet hemocyanin into rabbits, much of the antigen localized in the liver but subsequently left the liver and was found in the spleen and to a lesser degree in lymph nodes.<sup>99</sup> A comparison of the localization of carbon particles and <sup>125</sup>I-labeled salmonella antigens after intravenous injection showed different distribution in the spleen depending on the time of evaluation.<sup>122</sup> Antigen was first found in the marginal zone and red pulp, but then appeared to concentrate in the marginal zone and move across the marginal sinus and into the white pulp, finally localizing in the cap of the lymphoid follicles. A similar but slower and less intense accumulation of antigen occurred after subcutaneous foot-pad injection, most of the antigen remaining in the regional nodes. Carbon particles also were found first in the marginal zone and red pulp. Later they moved to the periarterial white pulp and then to "tingible body" macrophages in the germinal centers. In all three

studies it appeared that discrete, particle-laden cells were redistributed after initial localization. The significance of these observations in relation to antibody formation is not entirely clear except that localization in lymphoid follicle caps of both the spleen and lymph nodes is much more intense in previously primed than in unprimed animals.<sup>117,118</sup> In any case, an early event after antigen localization was morphologic differentiation with increased numbers of plasma cells appearing as early as 13 hours after antigen injection.<sup>74</sup> Antibody could be detected (plaque formation method) by 72 hours, increased to a maximum at 96 hours, and serum antibody levels reached peak values 72 hours after that.<sup>74</sup>

In summary, the spleen is clearly involved in antibody production but the degree of involvement appears to vary with the type of antigen, the site of injection and the amount given. The spleen does not appear indispensable to antibody formation since antibody is formed in asplenic subjects. However, a delay in appearance and lower peak antibody titers are noted after splenectomy.<sup>75,133,134,135</sup>

### *Control over Hematopoiesis*

Ferrata believed that the spleen normally exerts an inhibitory effect on bone marrow activity. This possibility has intrigued investigators for years and was inferred from the blood changes demonstrable after splenectomy. However, as other explanations for many of these postsplenectomy changes have been provided, evidence for a direct (humoral) control over hematopoiesis has dwindled. Thus there is no evidence that the spleen influences the enzymatic synthesis of hemoglobin, porphyrins, or stromal proteins. Also the suggestion that the spleen determines the age at which young erythrocytes are released from the bone marrow and that postsplenectomy reticulocytosis results from removal of this control is now explained in another way, namely, that the normal spleen traps reticulocytes and after splenectomy these sticky, easily agglutinated young cells can circulate freely in the blood.<sup>101,108</sup> On the

other hand, the concept that the spleen might influence the stem cell population in the bone marrow<sup>92</sup> has been expanded, and it is now clear that in mice the spleen may serve as a source of colony-forming units (cells) capable of repopulating a depleted marrow.<sup>86</sup> Also it has been suggested that homogenates of mouse spleen enhanced recovery from irradiation and increased the number of hematopoietic stem cells in the marrow.<sup>112</sup> That this may not represent specific control over hematopoiesis is suggested by the fact that an increase in hematopoietic stem cells as measured by endogenous spleen colony formation can be produced by injection of foreign plasma, endotoxin, and a variety of other substances.<sup>85</sup> Thus it remains to be demonstrated that there is a specific splenic humoral controlling substance.

There is evidence that the spleen may exert some control on leukocytes in that the leukocytosis that develops following splenectomy in normal animals is much more pronounced than that occurring after other operations.<sup>123</sup> It was shown that the usual postsplenectomy leukocytosis can be prevented by leaving 25% of the organ intact within the body, or by transplanting as little as 10% of a spleen that had been removed.<sup>123</sup> Furthermore, when splenectomy was performed in one partner of parabiotic rats, no rise occurred in the leukocyte count of either animal; only when the spleen of the second partner was removed did a rise in the leukocyte count take place and this occurred in both animals. That a splenic humoral factor may exist<sup>126</sup> is also suggested by observations of the effects of splenectomy and reimplantation of splenic tissue.<sup>142</sup> These observations suggest that the rate of production or liberation of leukocytes from the marrow may be under the control of the spleen, but other explanations are possible and no splenic humoral "leukopoietin" has yet been demonstrated. There is some evidence that the spleen is a source of granulocyte colony-stimulating activity, but not the sole source.<sup>137</sup>

The well-known observation that radiation directed to the spleen is associated with a reduction in circulating leukocytes in leuke-

mic patients has led to speculation that a humoral factor liberated by the spleen in response to roentgen therapy causes this decrease. Studies of the effects of the reinjection of plasma drawn before and after irradiation of the spleen provided support for this hypothesis.<sup>115</sup> However, attempts made from time to time to demonstrate inhibitory effects of splenic extracts on hematopoiesis have yielded only equivocal results.<sup>76,125</sup>

Postsplenectomy thrombocytosis, as already discussed (page 359), appears to be explainable on the basis of removal of the normal storehouse for about one third of the blood platelets.<sup>77</sup> No splenic thrombopoietic activity has yet been demonstrated.

Disorders of the spleen and methods for their study, including splenic puncture, are discussed in Chapter 45.

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## SECTION 4: Platelets, Hemostasis, and Coagulation

Hemostasis has been defined as "the process which spontaneously arrests the flow of blood from vessels carrying blood under pressure."<sup>244</sup> Few biologic processes are of more immediate and critical homeostatic importance. In all animals, including even the invertebrates, hemostasis is normally accomplished by a combination of three processes, ie, the contraction of the vessels, the adhesion and aggregation of formed blood elements such as the amebocyte or platelet, and the process of blood or plasma coagulation.<sup>244</sup> In man these vascular, cellular, and biochemical hemostatic functions have evolved to a high degree of complexity. All three are required for completely efficient hemostasis, but they are homeostatically independent to the extent that hemostasis compatible with life is maintained even if one component, such as the platelets or a single coagulation factor, is deficient.

Despite their physiologic importance, the processes of platelet aggregation and blood coagulation may constitute a threat to the organism if they propagate beyond the wound site. They normally are kept within desirable limits by various homeostatic control mechanisms. Once the function of the hemostatic barrier has been served, fibrin is removed by the fibrinolytic enzyme system and the leukocytes, a process which leads to recanalization of the damaged vessels.

In the next two chapters, hemostasis and the various homeostatic control mechanisms will be discussed under three headings, namely, the functions of the vessels, the platelets, and the coagulation mechanism. The events of the vascular and platelet phases, which together produce the first or temporary hemostatic barrier (*primary hemostasis*), will be considered in the following chapter (Chapter 9). *Secondary or permanent hemostasis* requires the formation of fibrin. This protein is the product of the coagulation phase, which, together with the fibrinolytic enzyme system, is discussed in Chapter 10.

These various phenomena will be considered in this volume mainly in terms of their role in hemostasis. However, with advancing knowledge it has become apparent that they are intimately related to other defense mechanisms, eg, vascular permeability, inflammation, wound healing, the action of the complement system and other immunologic reactions.<sup>172,291,294,298</sup>

Much of what has been learned about abnormalities of coagulation, platelet dysfunction, and quantitative variations of platelets in disease is pertinent to a knowledge of normal hemostasis and blood coagulation. Thus, the information presented in Part IV (page 1040) serves in many ways to supplement the following discussion.



## *Platelets and Megakaryocytes: The Physiology of Primary Hemostasis*

### **The Blood Platelet**

Morphology  
Biochemistry  
Origin

### **The Megakaryocyte**

Morphology and Histogenesis  
Mechanisms of Platelet Production  
Extramedullary Megakaryocytes

### **Kinetics of Thrombopoiesis**

Megakaryocyte Proliferation  
Platelet Distribution and Survival  
Regulatory Processes  
Physiologic Variations

### **The Vascular Phase of Hemostasis**

### **The Platelet Phase of Hemostasis**

Platelet Adhesion  
Platelet Aggregation  
Release Reaction  
Role of Cyclic AMP and Prostaglandins  
Morphologic Changes  
Role of Platelets in Blood Coagulation  
Consolidation Phase  
Clot Retraction

### **Homeostatic Control Mechanisms**

### **Miscellaneous Platelet Functions**

aspects of the role of the platelet in coagulation. The following information concerning the platelet is discussed elsewhere: its antigenic structure (Chapter 12), methods for *in vitro* preservation and the therapeutic use of platelet transfusions (Chapter 12), techniques for enumeration and for assessing various individual platelet functions (Chapter 33).

## **The Blood Platelet**

In the early part of the 19th century, many investigators observed the blood platelets,<sup>411</sup> but even Zimmermann (1860), Max Schultz (1865) and Osler (1874), who realized that these particles were not artifacts, failed to recognize their true importance. Hayem (1878), like Zimmermann, thought that they developed into red blood cells. It remained for Bizzozero<sup>42</sup> (1882) to describe platelets as they appeared in the mesenteric vessels of rabbits and guinea pigs. He demonstrated their adhesive quality, their participation in thrombi, and their role in the coagulation of the blood. The origin of the platelet from the megakaryocyte was established by the important studies of Wright.<sup>448</sup>

### **Morphology**

The morphology of platelets varies greatly depending on the methods by which they are examined, the anticoagulant, and the temper-

**T**HE present chapter will summarize information concerning the platelets, their precursor cell—the megakaryocyte, and the phenomena of primary hemostasis, including, for convenience, the events of the vascular phase. The discussion of platelet function will include the effects of the products of coagulation on platelets, and certain

ature.<sup>259</sup> In *wet preparations*, platelets are colorless, moderately refractile bodies that are discoid or elliptical in shape. Under dark-field illumination they are translucent, and reveal a sharp contour. A few immobile granules are present in the center of the cell. When observed by phase contrast microcinematography, contractile vacuoles and vacuoles of pinocytosis have been noted.

In *smears* stained by one of the Romanowsky methods, platelets appear round, oval, or rod-shaped. Azurophilic granules are seen in a hyaline, light-blue cytoplasm. These granules may be so tightly packed in the central portion of the platelet that they give the appearance of a nucleus. To the granular and hyaline portions the terms "granulomere" and "hyalomere" have been applied, respectively, but instantaneous fixation and staining with dyes like brilliant cresyl blue fail to show a sharp division into two portions. The platelet granules stain with neutral red; a few mitochondrial rods and granules stain with Janus green.

The dimensions of the platelet, as determined by electron microscopy, average 1.5  $\mu\text{m}$  in diameter and from 0.5 to 1  $\mu\text{m}$  in thickness.<sup>192</sup> The determination of platelet size by conventional microscopic techniques is fraught with many difficulties.<sup>259</sup> Estimates of platelet volume obtained by older "thrombocytocrit" methods<sup>330</sup> are erroneously high due to the marked platelet swelling which may occur rapidly in certain anticoagulants and at temperatures below 37°C.<sup>65</sup> As determined by means of an electronic particle counter, modal platelet volume ranges from 5 to 6  $\mu\text{L}$ ,<sup>363,364</sup> but it has been suggested that the assumptions upon which impedance counting are based may not be entirely valid in the case of platelets.<sup>52</sup>

A qualitative estimate of platelet volume, and thereby of their number, may be obtained by observing the thickness of the platelet layer in a hematocrit that has been centrifuged after sedimentation has taken place. If a hematocrit filled with blood is allowed to stand an hour or longer, the red cells settle to the bottom, the leukocytes settle more gradually, and the platelets remain suspended

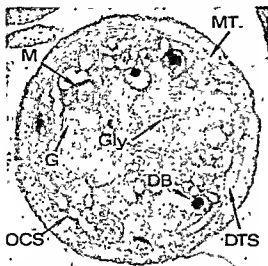
in the plasma. If centrifugation is then carried out, slowly at first and then more rapidly, three layers may be seen, namely, a creamy-white layer of platelets, a reddish-gray layer of leukocytes, and a red layer of erythrocytes (Plate 1, Frontispiece, and Fig. 3-20, page 112). This method serves only as a rough guide because some platelets remain in the plasma in spite of centrifugation and others are intermixed with the leukocytes.

### Ultrastructure

The platelet membrane is approximately 7.5 nm thick, and has a trilaminar unit structure<sup>53, 432</sup> (Fig. 9-1). Although it has been suggested that it is derived from endoplasmic reticulum of the megakaryocyte,<sup>53</sup> most evidence favors the view that it is a derivative of the plasma membrane of the parent cell, and thus resembles the external membranes of most other cells.<sup>22, 29</sup> External to the membrane is the *surface coat*, a "fuzzy," irregular layer ranging from 10 to 50 nm in thickness which is clearly revealed only by means of special fixation and staining procedures.<sup>432</sup> It is composed of a variety of proteins and mucopolysaccharides and contains a high concentration of sialic acids.<sup>313</sup> This coat may represent the adsorbed "plasmatic atmosphere"<sup>335</sup> or a true component layer of the cell membrane.<sup>463</sup>

The *marginal microtubular system* consists of 5 to 10 structures, approximately 20 nm in diameter, which encircle the periphery of the cell (Fig. 9-1). First revealed by the technique of double fixation,<sup>367</sup> these structures appear to contain thrombosthenin,<sup>462</sup> and under certain circumstances seem to undergo a reversible depolymerization into smaller microfibrils 7 nm in diameter.<sup>23, 423, 462</sup> The changes that occur in the microtubules during platelet aggregation and the location of these structures within the platelet suggest that they function as both the platelet "cytoskeleton"<sup>30, 428</sup> and the intrinsic contractile apparatus.<sup>28, 269, 366, 462</sup> A second microtubular system (the *dense tubular system*) is closely associated with the marginal microtubules.<sup>29</sup>

The *surface-connecting tubules* (canalicular



**Fig 9-1.** Ultrastructure of human blood platelets. *Left*, Thin section of human platelet. Mitochondrion (M), marginal microtubules (MT), dense tubular system (DTS), dense body (DB), glycogen (Gly), granule (G), canalicular system (OCS) (From White<sup>433</sup> courtesy of the author and the *Annals of the New York Academy of Sciences*) *Below*, Section of a platelet fixed after incubation in distilled water for five minutes at 37°C. Microfibrils have become apparent in the cytoplasm. Microtubules (arrow) are intact. Note the unit membrane and surface coat  $\times 60,000$  (From Zucker-Franklin,<sup>462</sup> courtesy of the author and the *Journal of Clinical Investigation*)



system) are a network of vesicles and interconnecting channels which ramify throughout the entire cytoplasm and communicate with the cell surface. This system is lined with the same membrane and surface coat that covers the external surface of the platelet, and is found in close apposition to storage organelles, eg, "dense bodies."<sup>432</sup> This and other observations<sup>136</sup> suggest that the release reaction may occur through openings formed by fusion of the limiting membrane of the

storage organelles with that of the external membrane and the canalicular system.<sup>433a</sup> This tubular system also appears to be involved in platelet phagocytosis (page 400). The cytoplasm of the platelet contains masses of microfilaments (Fig. 9-1).<sup>136,384,464</sup>

**Granules and other organelles** (Fig. 9-1) include small mitochondria with 2 to 3 cristae,<sup>192</sup> glycogen granules,<sup>371</sup> lipid-filled inclusions, ferritin granules ("siderosomes"), and, in an occasional platelet, a Golgi appara-

tus.<sup>338</sup> Platelet ribosomes are very difficult to identify microscopically, but have been demonstrated in small numbers in cell homogenates.<sup>398</sup> Various electron-dense granules also are present; these vary greatly in appearance depending on the technique of fixation. The *alpha granule* is oval in shape and 0.15 to 0.4  $\mu\text{m}$  in greatest diameter.<sup>192</sup> Its membrane structure and contained acid phosphatase suggest that it is a lysosome.<sup>269,432</sup> This hypothesis has been questioned,<sup>380</sup> but is consistent with alterations that have been observed in platelets during storage.<sup>131</sup> Laminar structures which resemble phospholipid micelles also have been demonstrated in the *alpha granules*, and may represent platelet factor 3 (page 397).<sup>435</sup> However, quite similar structures have been encountered in various degenerating cells.<sup>192</sup>

The *platelet dense body* (dark body, "bull's-eye" granule, "very" electron-dense granule) is a round structure, 0.05 to 0.15  $\mu\text{m}$  in diameter, which is often embedded within granular matrix of distinctly less electron density or may be seen within small vacuoles. These storage and secretory organelles appear to develop from undifferentiated platelet granules,<sup>269,432</sup> and contain nucleotides, calcium ions, catecholamines, and possibly platelet factor 4<sup>104,269</sup> (page 398). It is probable that some authors use the terms "*alpha granule*" and "*dense body*" interchangeably.

### Variations in Size and Morphology

Attempts have been made to classify platelets on the basis of size<sup>265</sup> and other criteria,<sup>210,329</sup> but these features are highly variable and difficult to quantitate by simple microscopic methods.<sup>259</sup> Methods based on electronic sizing<sup>65,364</sup> and density gradient centrifugation<sup>16</sup> are more promising, but a "differential" classification of platelets is, at best, fraught with much difficulty.

There is good evidence that young platelets are larger and more dense than older cells.<sup>11,213</sup> Thus, platelet anisocytosis and macrocytosis are present when platelet production is accelerated. The number of abnormally large platelets ( $>2.5 \mu\text{m}$  in diameter)

bears a direct relationship to the number of megakaryocytes, an observation which may be clinically useful<sup>140</sup> (Chapter 34). These changes may be marked in disorders characterized by accelerated platelet destruction, eg, autoimmune thrombocytopenias (Chapter 34). In such disorders, platelets with very coarse granules, "basophilic" platelets, and forms with diminished granulation have been reported. In neonates, there may be more variation in the size of platelets and in their staining reaction than is normal in adults. In dogs, "reticulated" platelets have been demonstrated following acute blood loss.<sup>200</sup>

Large and bizarre platelets often are present following splenectomy (page 359), in myelofibrosis (Chapter 57), hemorrhagic thrombocythemia (Chapter 34), and in polycythemia vera (Chapter 30). Certain disorders of platelet function associated with thrombocytopenia are characterized by particularly large "giant" platelets (Fig. 35-3, page 1126). In other disorders, normal numbers of totally agranular platelets or abnormally small platelets may be seen (Chapter 35).

All of these changes in platelet morphology must be distinguished from very similar artifacts which arise in poorly collected blood specimens and in stored anticoagulated specimens.

### Biochemistry of the Platelet

From an "inert particle" that at one time was even thought to be an artifact, the platelet has emerged as a most remarkable structure which possesses abundant metabolic equipment, including more than 80 different enzymes,<sup>95,239</sup> significant synthetic capability, a capacity to expend considerable energy, and, like muscle, the ability to contract when appropriately stimulated.<sup>207</sup>

The chemical composition of the platelet (Table 9-1 and page 388) is difficult to determine accurately because of the tendency of this cell to adsorb a variety of substances from the plasma, the fact that centrifugation and washing may induce rapid and irreversible biochemical changes, and because of variations in its biochemical properties which depend on the age of the cell.



In terms of dry weight, the platelet is composed of approximately 60% protein, 15% lipid, and 8% carbohydrate. Important minerals include Mg,<sup>454</sup> Ca,<sup>454</sup> K,<sup>454</sup> and Zn.<sup>133</sup>

Copper and the heavy metals are absent.<sup>259</sup> Platelets contain substantial amounts of vitamin B<sub>12</sub>, folic acid, and ascorbic acid.<sup>425</sup>

Table 9-1. Some Quantitative Biochemical Data on Human Platelets

	Amount (/10 <sup>9</sup> Platelets)	Percentage	Reference
I ELEMENTAL			
Dry weight	2.8 mg	—	
Nitrogen total	0.31 mg	11.0*	
Nitrogen protein	0.255 mg	9.0*	
Phosphorus total	—	1.15*	
Sulfur total	—	0.18*	
Carbohydrate total	0.238 mg	8.5*	
Protein total	1.6 mg	57.0*	
Lipid total	0.53 mg	19.0*	
II CARBOHYDRATES			
Polysaccharides total	0.393 mg	—	
Hexoses	—	4.41*	447
Glycogen	0.065 mg	2.34*	331
Pentoses	—	0.67*	447
Sialic acids	—	0.72*	447
Galactosamine	—	2.67*	447
Mucopolysaccharides	0.136 mg	—	
III LIPIDS			
Phospholipid	—	74-77†	250
Neutral lipid	—	20.8†	
Free cholesterol	—	18.7†	253
Proteolipid protein	—	1.8†	
Ganglioside	—	0.5†	
Lysophosphatidylcholine	—	1.7†	77
Diacyl choline phosphoglycerides	—	39.8†	
Sphingomyelin	—	18.4†	
Diacyl ethanolamine phosphoglycerides	—	13.6†	
Plasmalogen ethanolamine phosphoglycerides	—	14.0†	
Plasmalogen choline phosphoglycerides	—	<1.0†	
Inositol phosphoglycerides	—	3.7†	
Serine phosphoglycerides	—	8.8†	
IV PROTEINS			
Thrombosthenin	—	12-20§	34, 302
Fibrinogen	—	5-13.5§	36
V NUCLEOTIDES			
Adenosine triphosphate	71 nM	—	275
Adenosine diphosphate	40 nM	—	
Adenosine monophosphate	5 nM	—	
Cyclic AMP	140 pM	—	365
VI MISCELLANEOUS			
Taurine	—	0.15-0.2§	
5-Hydroxytryptamine	0.2-0.57 µg	—	
Histamine	0.1 µg	—	
Catecholamines, total	16-33 µg	—	

\* of platelet dry weight

† of total platelet lipid

‡ of total phospholipids

§ of total platelet protein

Except where cited, data obtained from Maupin<sup>259</sup>

The sodium and potassium concentrations within the platelet are 39 and 138 mEq, respectively.<sup>147</sup> This gradient against plasma is maintained by an active ion pump, which derives energy from a membrane adenosine triphosphatase of the ouabain-sensitive,  $\text{Na}^+/\text{K}^+$ -dependent type.<sup>81,147</sup>

### Energy Metabolism

The similarities between the energy metabolism of the platelet (Fig. 9-2) and that of skeletal muscle have been emphasized repeatedly.<sup>216</sup> Both involve active glycolysis and the synthesis and utilization of large amounts of glycogen,<sup>218</sup> and in both the major mediator of intracellular energy utili-

zation is an actomyosin-like adenosine triphosphatase. The platelet, like muscle, is metabolically adapted to expend large amounts of energy rapidly, ie, during aggregation, the release reaction, and clot retraction.

The major energy source for the platelet is glucose, which is rapidly taken up from the plasma (Fig. 9-2). Under basal conditions, 40 to 50% of the absorbed glucose is used to provide energy for synthetic functions or is converted into glycogen,<sup>212</sup> which provides an endogenous store of carbohydrate.<sup>215</sup> Fatty acids also are metabolized.<sup>79,112</sup>

The glycolytic pathway with its regulatory enzymes (phosphorylase,<sup>217</sup> hexokinase,<sup>357</sup> phosphofructokinase<sup>107</sup>), the tricarboxylic

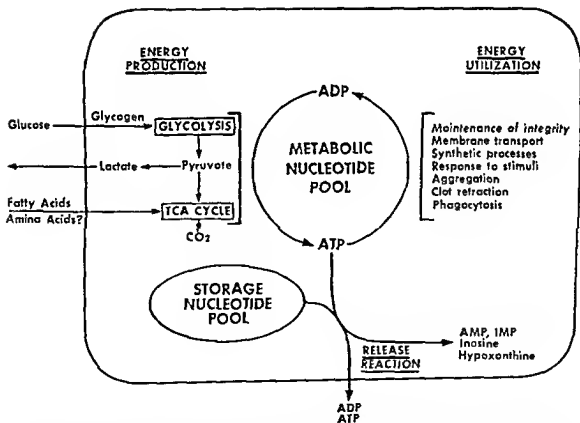


Fig 9-2. Simplified scheme of platelet energy metabolism. Platelet energy is derived from the metabolism of glucose and to a lesser extent from the metabolism of fatty acids. Energy is provided in approximately equal amounts by glycolysis and the tricarboxylic acid cycle. The platelet energy reserve is provided by the metabolic pool of platelet nucleotides that are in a state of continuous turnover. This energy is utilized for the maintenance of the platelets' structural integrity and in the reactions accompanying the response of platelets to stimuli. The granule-bound storage (nonmetabolic) nucleotide pool is discharged during the release reaction. (From Hirsh and Doery<sup>122</sup> courtesy of the authors and Progress in Hematology.)

acid cycle, the pentose-phosphate pathway, and the NAD-NADH system<sup>74</sup> are all active in the platelet.<sup>239</sup> Under normal circumstances, glycolysis is more active than oxidation, but the total ATP synthesized by the two pathways is approximately equal because of the greater ATP yield per mole of glucose provided by the latter.<sup>112, 212</sup> "Stimulation" of the platelet by agents that induce aggregation and the release reaction is associated with a marked increase in metabolic activity involving glycogenolysis and both glycolytic and oxidative pathways to variable degrees.<sup>172, 212, 231, 456</sup>

The ATP content of the platelet is 150 times that of the red cell (Table 9-1). Adenine nucleotides comprise 90% of free platelet nucleotide, and are synthesized from both adenine and adenosine. The platelet lacks the capacity for *de novo* nucleotide synthesis.<sup>181</sup> During the release reaction, approximately 50% of the platelet nucleotides are lost from the cell without impairing its metabolic viability. This is due to the fact that platelet nucleotides are partitioned into at least two different pools (Fig. 9-2).<sup>178, 184</sup> The *metabolic pool* is utilized for the maintenance of the various energy-consuming cell functions. Comprised largely of ATP, this pool is constantly turning over, as revealed by the rapid incorporation of <sup>14</sup>C-adenine and <sup>32</sup>P-phosphate into ATP.<sup>178</sup> The *storage pool* contains both ADP and ATP, is metabolically inactive, and does not incorporate exogenous adenine or phosphate. Nucleotides in this pool are extruded from the cell during the release reaction. There is preliminary evidence that the nucleotides in the storage pool are bound to thrombosthenin.<sup>185</sup>

The ATP that is broken down to provide energy for the release reaction is not rephosphorylated but, rather, is degraded (Fig. 9-2), an observation suggesting that both high-energy bonds have been utilized.<sup>184</sup> This ATP may be compartmentalized in a third pool that is in equilibrium with the metabolic pool.<sup>184</sup>

The role of cyclic AMP in the regulation of platelet function and energy metabolism

is discussed on page 395. The energetics of clot retraction are discussed on page 399.

## Proteins

The platelet has been likened to a sponge<sup>7</sup> because of its remarkable propensity to adsorb various substances from the plasma. These include proteins, amino acids,<sup>433</sup> histamine,<sup>269</sup> ions, and various drugs.<sup>292</sup>

Twenty *amino acids* have been identified in the platelet.<sup>259</sup> Of these, glycine, glutamic acid, alanine, serine, and aspartic acid are present in the highest concentrations.<sup>150, 259</sup> Taurine is present in concentrations averaging 200 times those of plasma (Table 9-1); its physiologic significance is uncertain.<sup>269</sup>

As many as 20 different proteins have been demonstrated in platelet homogenates by means of polyacrylamide gel electrophoresis,<sup>240</sup> but the differentiation between those that are nonspecifically adsorbed and those that are intrinsic to the cell is often difficult. Several have been identified immunologically, including thrombosthenin, albumin, prealbumin, IgG and IgM globulins,<sup>300</sup> plasminogen,<sup>99</sup> and fibrinogen.

*Fibrinogen*, in both an adsorbed and a granule-bound form, has been demonstrated in platelets.<sup>69, 149</sup> This protein also has been demonstrated in the megakaryocyte.<sup>145</sup> Data suggesting that platelet fibrinogen is biochemically different from plasma fibrinogen<sup>99, 138a</sup> have been disputed.<sup>94, 139</sup> Fibrinogen is an essential cofactor for ADP-induced platelet aggregation (page 391) and possibly is involved in other platelet functions.<sup>374a</sup>

Virtually all of the other *plasma coagulation factors* have been demonstrated in association with the platelet.<sup>172</sup> The vitamin K-dependent factors (prothrombin, factors VII, IX and X) appear to be weakly adsorbed and are readily removed by washing.<sup>108, 199</sup> whereas factors V, VIII,<sup>211</sup> XI, and XII<sup>189, 199</sup> appear to be more firmly bound. As much as 50% of the factor XIII in blood is associated with the platelets, and there is convincing evidence that this factor is synthesized by

the megakaryocyte and is not adsorbed from the plasma.<sup>261</sup>

There is much to suggest that the proteins present on the surface of the platelet ("*the plasmatic atmosphere*") and their interactions with those which coat vascular surfaces are important in platelet function.<sup>21,362</sup> Fibrinogen, as discussed above, factor XII,<sup>247</sup> and other as yet unidentified proteins<sup>90,110</sup> appear to have specific functional importance in platelet aggregation and in the release reaction. The interaction of the platelet with synthetic surfaces and particles can be specifically conditioned by various protein coatings.<sup>263,296,336,439</sup> Preliminary experiments with carefully washed platelets suggest that a balance between adsorbed anionic proteins and various cationic proteins, such as IgG immunoglobulin, factor XIIa, and fibrinogen, may be important in maintaining an optimal charge at the platelet surface.<sup>21</sup>

Platelets are able to synthesize both amino acids and proteins.<sup>423</sup> <sup>14</sup>C-labeled acetate and glucose are rapidly incorporated into glutamic acid, aspartic acid, glutamine, and asparagine,<sup>350</sup> while isotopically labeled amino acids are rapidly incorporated into platelet proteins, including fibrinogen<sup>85</sup> and thrombosthenin.<sup>399</sup> Protein synthesis takes place principally in young platelets.<sup>46,433</sup> These observations are noteworthy, since the platelet contains negligible DNA and the RNA present is in a metabolically inactive form. It has been suggested that protein synthesis is the result of residual stable messenger RNA<sup>45,46</sup> or possibly occurs in the mitochondria.<sup>151</sup>

**Thrombosthenin**, a contractile protein complex comprising 15 to 20% of total platelet protein, can be dissociated into two fragments which resemble actin (*thrombosthenin A*) and myosin (*thrombosthenin M*) of skeletal muscle.<sup>34,35</sup> The subunit molecular weights of these two fragments have been estimated at 46,000 and 200,000, respectively.<sup>9</sup> Under the electron microscope, thrombosthenin A appears as a rod-shaped molecule approximately 6 nm long and 0.1 to 0.2 nm thick.<sup>31</sup> It reveals an axial periodicity of 3.5 nm.

Thrombosthenin M is composed of large spindle-like structures, 0.2 to 0.5 nm long,<sup>31</sup> that presumably are polymeric in nature.<sup>35</sup> The "thrombin-sensitive" protein of the platelet membrane apparently is identical to thrombosthenin M.<sup>20,47</sup> Detailed studies of thrombosthenin M have revealed functional properties that are identical with myosin purified from skeletal muscle.<sup>9</sup> This evidence would seem to justify the conclusion that thrombosthenin is identical to the actomyosin system of muscle, as was first hypothesized many years ago.

In platelet homogenates, thrombosthenin is found almost entirely in the membrane and granule fraction.<sup>302</sup> Ultrastructural studies would suggest that it is associated with the marginal microtubular system<sup>35,68</sup> and perhaps with other organelles as well.<sup>137</sup> Quantitative assays of thrombosthenin by means of highly sensitive radial immunodiffusion techniques have revealed two fractions. That present throughout the cytoplasm (*thrombosthenin C*) comprises 90% of the total, while the remainder is located on the surface of the platelet (*thrombosthenin S*). Both of these fractions have adenosinetriphosphatase activity of the  $Mg^{++}/Ca^{++}$ -dependent type. Adenosinetriphosphatases which differ from thrombosthenin also are present in the platelets.<sup>302,431</sup>

The contraction of thrombosthenin underlies the phenomenon of clot retraction, and may explain the initial change in platelet shape produced by ADP, as well as the "cohesion" of secondary aggregates. It may also be involved in platelet aggregation (page 396).

### Lipid Metabolism

Platelets synthesize both fatty acids and phospholipids. <sup>14</sup>C-acetate and palmitate are incorporated into various platelet lipids.<sup>126</sup> <sup>432</sup> A high percentage of the label is initially present in free fatty acids in the platelet membrane<sup>432</sup>; these are synthesized both *de novo*<sup>435</sup> and by means of chain elongation, and are freely exchangeable with plasma fatty acids.<sup>109</sup> Phospholipids comprise 80% of the

total platelet lipid<sup>250</sup> (Table 9-1). Both <sup>14</sup>C-labeled fatty acids<sup>76</sup> and <sup>32</sup>P-phosphate<sup>312</sup> are incorporated into platelet phospholipids.<sup>397</sup> The turnover rate of these compounds, which normally is relatively slow, is significantly accelerated when platelets are "stimulated" by collagen, ADP, or thrombin.<sup>238a,312</sup> The phosphatidyl inositols, in particular, are heavily labeled under these circumstances, as in leukocytes during phagocytosis.<sup>312</sup> Significant concentrations of arachidonic acid, the precursor of prostaglandin PGE<sub>2</sub>, also have been demonstrated in the platelet.<sup>111</sup>

The role of platelet lipids in blood coagulation is discussed on page 397.

### Carbohydrates

The carbohydrates of platelets consist mainly of glycogen (Table 9-1). Heterosaccharide complexes containing sialic acids are abundant in the platelet membrane and surface coat,<sup>246,343</sup> and are important determinants of the surface charge. They also serve as acceptor sites for enzymes that may be involved in platelet adhesion<sup>202</sup> (page 391). Small amounts of glycolipids (gangliosides) also are present.<sup>254</sup> These compounds, and the sialoglycoproteins, are probably of importance in receptor sites for active transport functions.<sup>246</sup> Sulfated mucopolysaccharides<sup>319</sup> and a number of monosaccharides<sup>417</sup> are present as well.

### Origin of the Platelet

J. H. Wright concluded in 1906 that platelets are detached portions of the cytoplasm of megakaryocytes.<sup>418</sup> Wright's observations in cats were confirmed in other mammals by Bunting,<sup>66</sup> Downey,<sup>113</sup> and others,<sup>235</sup> and his theory is now generally accepted.<sup>226</sup> More recent evidence for this hypothesis may be summarized as follows: (1) platelets are first observed in the embryo at about the same time as megakaryocytes appear; (2) experimentally induced thrombocytosis is associated with an increase in the number of megakaryocytes; (3) there are numerous simi-

larities between the cytochemical<sup>239,410,450</sup> and antigenic<sup>418</sup> composition of megakaryocytes and platelets; (4) radioactive isotopes that are incorporated into megakaryocytes are subsequently found in the platelets; and (5) platelet production from megakaryocytes has been observed directly in animals.<sup>33,171,225</sup>

## The Megakaryocyte

### Morphology and Histogenesis

Megakaryocytes (Plate VIII, page 382; Fig. 9-3) are exceedingly large cells ranging from 35 to 160  $\mu$ m in diameter. They contain an irregularly lobed, ring- or doughnut-shaped nucleus composed of dense chromatin and staining brilliant blue with Wright's stain. Nucleoli are not seen. The cytoplasm is abundant and light blue, and is packed, except for a narrow rim at the periphery, with fine azurophilic granules. In the fetus, megakaryocytes are found successively in the liver, spleen, and bone marrow. In adult mammals they are precot in the bone marrow, lung, and spleen.<sup>153</sup>

The identification of normal megakaryocytes seldom is difficult, but occasionally these cells may be confused with other giant cells (Plate II, page 72), including those that are normally present in the marrow in small numbers (osteoclasts, osteoblasts, reticulum cells, rarely multinucleated erythroblasts and plasma cells), and pathologic cells (tumor cells, Reed-Sternberg cells, "storage cells").<sup>352</sup>

### Origin

Megakaryocytes have been considered to arise from a number of sources including primitive mesenchymal cells (Maximow), reticulum cells (Sabin), hemocytoblasts or large lymphocytes,<sup>410</sup> and from cells lining the sinusoids of the liver.<sup>5</sup> However, the evidence is now convincing that they originate, in common with erythrocytes and granulocytes, from the pluripotential stem cell. This is discussed on page 49.



Fig. 9-3 Megakaryocyte in material aspirated by sternal puncture Wright's stain  $\times 650$ . A metamyelocyte and a large normoblast as well as erythrocytes are shown for comparison of size.

### Development

The first morphologically recognizable precursor is the *megakaryoblast* (Plate VIII, page 382). This cell is 15 to 50  $\mu\text{m}$  in diameter and contains a large, oval or kidney-shaped nucleus with several nucleoli. The cytoplasm is scanty, nongranular, and intensely basophilic. Mitoses may be seen. The megakaryoblast enlarges, cytoplasmic granules begin to appear, and the *promegakaryocyte*, a cell 20 to 80  $\mu\text{m}$  in diameter, is formed. The nucleus of this cell is oval or irregular in shape; the cytoplasm is more abundant than that of the megakaryoblast and contains granules that develop first in the perinuclear zone. From this precursor, the *mature granular megakaryocyte*, described above, is formed. The terms *stages I, II and III* are often used with reference to the degree of maturity of megakaryocytes, and correspond, in general, to the designations megakaryoblast, promegakaryocyte, and granular megakaryocyte, respectively.

A qualitative assessment of the number of megakaryocytes relative to other marrow elements usually is sufficient for clinical purposes. This is done most quickly by scanning the smear with the microscope's low-power lens, which will clearly reveal these giant

cells. Megakaryocytes usually are most numerous in bone marrow "particles," and the number normally present in a bone marrow smear varies greatly depending on the relative cellularity of the specimen. The estimation of megakaryocyte numbers in hypocellular specimens is difficult. Quantitative methods for megakaryocyte enumeration are discussed below.

### Variations in Megakaryocyte Morphology

The morphology of the megakaryocyte normally is quite variable in stained smears. Some observers distinguish "reserve" megakaryocytes from platelet-producing forms on the basis of the absence of granules in the periphery of the cell.<sup>352</sup> Apparent "platelet production" from immature or nonfunctioning megakaryocytes may be due to artifacts, eg, "pseudothrombocytes" formed by shearing of a viscous cell coating during preparation of the smear,<sup>370</sup> the adhesion to the megakaryocyte of platelet aggregates formed from contaminating blood. Degenerating megakaryocytes, often surrounded by neutrophils, occasionally are seen in normal marrow.

When platelet production is accelerated,

megakaryocytes increase both in size and in number. In various forms of thrombocytopenia caused by accelerated platelet destruction, one may see immature forms that are not present in normal marrow, as well as morphologically abnormal and degenerating megakaryocytes and megakaryocytes that do not appear to be producing platelets (Plate XIV, page 1082). A differential count of megakaryocytes may have some value in such disorders,<sup>352</sup> which are discussed further in Chapter 34.

Abnormally large, hyperlobulated megakaryocytes with thin nuclear chromatin have been described in disorders caused by deficiency of folic acid or vitamin B<sub>12</sub><sup>352</sup> (Chapter 14). Numerous morphologically abnormal megakaryocytes often are present in myelofibrosis (Chapter 57). These are best seen in marrow specimens obtained by biopsy.

### Mechanisms of Platelet Production

Megakaryocytes are situated in the bone marrow in close proximity to the sinusoidal membrane.<sup>136</sup> Platelet production may occur by two somewhat different processes, both of which have been demonstrated in man.<sup>400</sup> Pseudopodial platelet formation, lucidly described by Wright,<sup>448</sup> has been demonstrated in several species by more modern techniques, eg, microcinematography in bone "windows" of rabbits.<sup>32</sup> Platelet formation begins by the development of numerous long cytoplasmic pseudopods that extend through apertures in the sinus membrane into the lumen, much in the manner of an octopus. These pseudopodia become thinner and ultimately filiform, and granular masses the size of platelets form within them. These processes probably are caught in sinusoidal capillaries, segments are broken off by their contraction, and the platelets thus formed are swept away by the blood stream.

Platelets are also formed by the *in situ* fragmentation of large portions of megakaryocyte cytoplasm. As visualized by electron microscopy,<sup>419</sup> this begins with the formation of "demarcation zones." These first appear as a chain of small vesicles that fuse to form fissures. The simultaneous widening

and fusion of these fissures result in the splitting of the megakaryocyte cytoplasm into 2000 to 4000 discrete units, the platelets. This process may involve large masses of cytoplasm simultaneously, or may occur by means of continuous demarcation of smaller areas.<sup>68</sup> Virtually the whole cytoplasm of the megakaryocyte is eventually broken away, and the bare nucleus remains to degenerate.

### Extramedullary Megakaryocytes

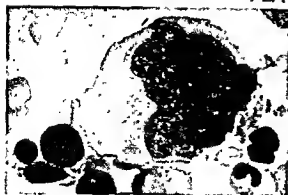
#### Lungs

It has been generally assumed that the main site of platelet production during adult life is in the bone marrow. Although megakaryocytes are normally found in the lungs, it was assumed that these were effete cells. Howell and Donabue<sup>196</sup> challenged this view on the following grounds: (1) the platelet count generally is higher in arterial than in venous blood; (2) fewer platelets are found in the bone marrow than in the spleen or lungs; (3) perfusion of bone marrow yields few platelets whereas perfusion of the lungs produces many; (4) the megakaryocytes observed in the lungs appear to be actively producing platelets. They concluded that platelets are normally formed in the lungs from megakaryocytes which develop there in the same manner as in the bone marrow. Although the formation of megakaryocytes in the lungs has not been substantiated, there is considerable support for the claim that platelets are released in the lungs from megakaryocytes carried there in the venous blood from the bone marrow.<sup>221</sup> As many as 20 to 50% of the mature megakaryocytes may enter the blood, ultimately reaching the lungs, and 7 to 17% of the body's platelets may be produced there.

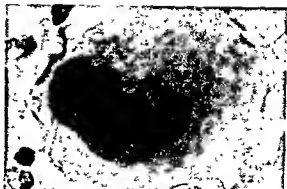
#### Blood

Megakaryocytes, or fragments of their nuclei or cytoplasm ("dwarf" megakaryocytes), are sometimes found in the circulating blood. These are of variable size and oval or irregular in shape. The nuclei stain deeply and may be surrounded by a small amount

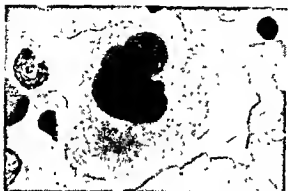
# PLATE VIII



A



B



C



D



E



F

*Megakaryocytes* (Wright's stain,  $\times 1000$ ) A, Megakaryoblast, B, promegakaryocyte, C, mature megakaryocyte, D, megakaryocyte shedding platelets, E, dwarf pro(?)megakaryocyte in blood, F, dwarf megakaryocytes in blood (myelofibrosis)



of cytoplasm.<sup>146</sup> These fragments are rarely seen in routine peripheral blood smears, but are found regularly in the blood of normal individuals when concentration techniques are used.<sup>61,267</sup> Megakaryocyte fragments may be seen even in routine blood smears in various forms of cancer,<sup>191</sup> myelophthisic processes, chronic myelocytic leukemia, polycythemia vera, Hodgkin's disease, following surgical procedures, chest injury, and cardiac massage, and when there is leukocytosis due to infection.<sup>277</sup> They are seen less commonly in aleukemic leukemia, thrombocytopenic purpura, pernicious anemia, and plumbism.

## Kinetics of Thrombopoiesis

### Megakaryocyte Proliferation

Morphologically recognizable cells of the megakaryocyte line lack the capacity for self-

renewal, and are maintained by a continuous influx of precursor cells from the stem cell compartment. There is good evidence that an intermediate "multiplicative" pool of stem cells is "committed" to differentiate into megakaryocytes.<sup>116,119,391</sup> The maturation of these primitive cells into mature megakaryocytes involves a unique process<sup>204</sup> (Fig. 9-4).

*Maturation of the nucleus* involves nuclear endoreduplication (endomitosis), a process in which nuclear material reduplicates but the nucleus does not divide. This results in a polyploid nucleus as distinguished from a polyploid cell, such as the osteoclast. Each division produces a doubling of the total nuclear material. This eventuates in a series of cells containing the equivalent of 4, 8, 16, and 32 sets of chromosomes; this number also is referred to as the nuclear number (N), "ploidy" value, or class.

*Maturation of the cytoplasm*, manifested by

## MEGAKARYOCYTOPOIESIS

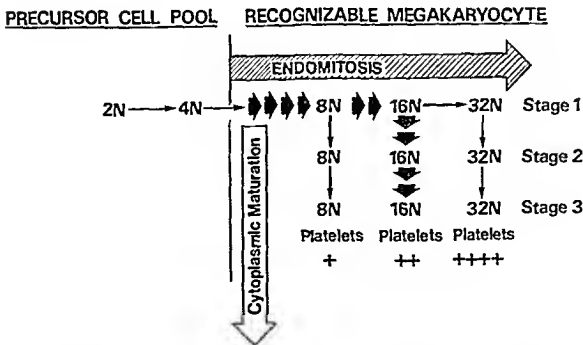


Fig. 9-4. Theoretical model of megakaryocytopoiesis. The earliest recognizable megakaryocyte is the  $8N$  cell. The wide arrows indicate the major pathway of nuclear and cytoplasmic maturation. The relative platelet yield per megakaryocyte is indicated by the + signs. (Based on the work of Odell et al.<sup>320,321,322</sup> From Hirsh and Doery<sup>177</sup> courtesy of the authors and *Progress in Hematology*.)

a progressive increase in its amount and granularity, and a loss of primitive basophilia, proceeds without cytoplasmic division. As judged by light microscopy, cytoplasmic maturation becomes apparent only after nuclear division has been completed. However, the development of cytoplasmic organelles is apparent much earlier in electron photomicrographs.<sup>338</sup> Both nuclear and cytoplasmic maturation result in an increase in the volume of the cell, ie, the largest megakaryocyte is the 32N granular (stage III) form. As estimated from DNA labeling in rats, the total megakaryocyte maturation time is approximately 60 hours<sup>118</sup>; in man an approximate figure of five days has been reported.<sup>92</sup>

Studies of the incorporation of <sup>3</sup>H-thymidine into the megakaryocytes of rats suggest that the primitive precursor is a 2N cell. Neither this cell nor the 4N cell is morphologically identifiable.<sup>118, 117, 119</sup> The mean nuclear number in the rat is 16N, but 8N and 32N cells are found in all stages of cytoplasmic maturation.<sup>327</sup> In man, 65% of the recognizable megakaryocytes in one study were of the 8N type as judged by microscopic nuclear counts.<sup>161</sup> With this method, a mean nuclear number of 8 was also found in the rat.<sup>158</sup> In view of evidence that DNA synthesis is confined to megakaryoblasts or even earlier precursors, it is probable that changes in nuclear lobulation associated with later maturation are unrelated to nuclear reduplication.<sup>321</sup> Nuclear lobe counts may be inaccurate as a means for the assessment of true ploidy value.<sup>118, 278</sup>

### Quantitation of Megakaryocytopoiesis

Since the platelet is produced by fragmentation of the cytoplasm of the megakaryocyte, the total number of platelets produced depends on the number and cytoplasmic volume of these precursor cells. Various methods for the enumeration of megakaryocytes have been described,<sup>344, 413</sup> none of which is entirely satisfactory. The one most widely used is an indirect method involving the microscopic determination of the number of megakaryocytes relative to the number of

erythroid precursors; the latter then are quantified with some degree of accuracy by labeling with radioactive iron. With this method, megakaryocytes normally number  $6.1 \pm 0.7 \times 10^6/\text{kg}$  body weight in man.<sup>156, 161</sup>

*Megakaryocyte volume* is difficult to determine accurately. Calculations based on surface area as measured in stained marrow smears<sup>156</sup> yield a mean figure of  $4200 \pm 100 \mu\text{l}$ .<sup>161</sup> For purposes of quantitation, an additional unit may be used, ie, the nuclear unit (the mean megakaryocyte volume divided by its nuclear number). This averages  $520 \mu\text{l}/\text{nucleus}$ .<sup>161</sup> The product of the total number of megakaryocytes and their mean volume is the *megakaryocyte mass*, which averages  $2.8 \pm 0.3 \times 10^{10} \mu\text{l}/\text{kg}$  body weight.<sup>161</sup>

When platelet production is stimulated, the megakaryocyte mass and total platelet production may increase as much as eightfold,<sup>159, 161</sup> as demonstrated by studies of patients with thrombocytopenia caused by accelerated platelet destruction. This results from a combination of accelerated nuclear reduplication, more rapid maturation, and, after an initial lag, increased input from the precursor compartment.<sup>160</sup>

### Platelet Distribution and Survival

#### Radioisotopic Techniques for Platelet Labeling

Among the isotopes presently available, <sup>51</sup>Cr-chromate and <sup>32</sup>P-diisopropyl-fluorophosphate are the most widely used for platelet labeling. The chromate ion binds to adenine nucleotides, and to various platelet proteins including thrombosthenin.<sup>400</sup> It is useful in man only as an in vitro whole population label. Low specific activity limits its usefulness in the study of thrombocytopenic subjects, although various technical modifications may obviate this difficulty.<sup>4</sup> DF<sup>32</sup>P is firmly bound by serine residues in platelet enzymes, is useful as either a cohort or total population label, and can be used both in vivo and in vitro.<sup>41</sup>

Neither DF<sup>32</sup>P nor <sup>51</sup>Cr-chromate are entirely satisfactory as platelet labels, how-

ever.<sup>172</sup> Both isotopes appear to label a predominantly young population of platelets<sup>213</sup>, both irreversibly damage or produce reversible sequestration of a small but possibly non-homogeneous population of cells<sup>41,172</sup>; and, in high concentrations, both compounds impair platelet function.<sup>219,297</sup> DF<sup>32</sup>P may prolong platelet survival,<sup>71</sup> and some <sup>32</sup>P released from effete platelets is recycled into circulating platelets.<sup>83</sup> Data suggesting that DF<sup>32</sup>P labels the megakaryocyte<sup>293</sup> have been disputed.<sup>83,121</sup>

Compounds such as <sup>75</sup>Se-selenomethionine<sup>301</sup> and <sup>35</sup>S-sulfate<sup>288</sup> are incorporated first into megakaryocytes and thence into circulating platelets, and consequently are useful as cohort labels in animals. <sup>75</sup>Se-selenomethionine is reincorporated into megakaryocytes, a significant disadvantage.<sup>301</sup> Because of rapid recycling of radioactivity, <sup>32</sup>P-phosphate<sup>152</sup> and <sup>14</sup>C-5-hydroxytryptamine<sup>170</sup> are no longer used as platelet labels.

EDTA is an unsuitable anticoagulant for *in vitro* platelet labeling since it markedly alters initial *in vivo* platelet distribution and recovery.<sup>16</sup> Most *in vitro* labeling techniques now employ acid-citrate-dextrose (ACD) additionally acidified to yield platelet-rich plasma of p<sub>H</sub> 6.5, a modification that minimizes platelet clumping.<sup>16</sup>

### Platelet Distribution *In Vivo*

When <sup>51</sup>Cr-chromate was used as an *in vitro* total population label, the initial *in vivo* recovery of labeled platelets averaged 65% in normal persons but was 90% or more in asplenic individuals.<sup>13,161</sup> Approximately two thirds of the total platelet mass thus appeared to be present in the circulation, while one third was in the spleen. This estimate has been confirmed by *in vitro* perfusion methods.<sup>312</sup> In the spleen, platelets apparently are sequestered within the sinuses or between the cells of the pulp. This splenic pool normally is interchangeable with the circulating platelet pool, and contains a disproportionately high percentage of young platelets.<sup>378</sup>

There are few data concerning the quanti-

tative significance of platelets produced by pulmonary megakaryocytes or of platelets present in the pulmonary vessels.<sup>221</sup> Judging by the rapidity with which thrombocytapheresis produces thrombocytopenia, platelet "reserves" in the lungs and other such extravascular sites appear to be minimal.<sup>38,87,258,377</sup> Platelets are not normally present in lymph or other body fluids.

### Life Span and Turnover Rate

Platelet life span, as estimated by *in vitro* whole population labeling with <sup>51</sup>Cr-chromate, ranges from 9 to 12 days in man.<sup>16,161</sup> Virtually identical values were obtained when DF<sup>32</sup>P was used as an *in vivo* whole population label.<sup>41,223</sup> Although the contour of the survival curves differed significantly, comparable figures for platelet lifespan also were obtained with <sup>75</sup>Se-selenomethionine and <sup>35</sup>S-sulfate.<sup>320</sup> Similar survival times were obtained with canine<sup>8</sup> and bovine<sup>279</sup> platelets, whereas platelet lifespan in rabbits,<sup>280</sup> mice,<sup>323</sup> and pigs<sup>354</sup> normally is shorter than in man.

In the steady state, platelet destruction is exactly balanced by platelet production, and an estimate of the number of platelets destroyed and replenished each day can thus be obtained by dividing the circulating platelet mass by the survival time in days. When appropriate corrections are made for variations in the initial recovery, a reasonably reproducible value for platelet turnover rate may be derived. This normally is  $35,000 \pm 4,300$  platelets/ $\mu$ l/day.<sup>161</sup>

### Fate of the Platelet

There is substantial clinical and experimental evidence that damaged or effete platelets are sequestered principally in the spleen<sup>13,11</sup> (Chapter 36). Vigorous controversy persists as to the major determinants of normal platelet survival and involves, for the most part, the interpretation of survival curves obtained with isotopically labeled platelets.<sup>11,96,293,298</sup> Rectilinear survival curves would be expected if the fate of the

platelet were determined mainly by senescence; curvilinear or first-order plots would be seen if the major factor were destruction by random processes unrelated to age.

In the majority of survival studies employing  $^{51}\text{Cr}$  as a total population label,<sup>16,161,238</sup> the plot was approximately rectilinear but deviated at the end, producing a "tail." Studies using  $\text{DF}^{32}\text{P}$  as an *in vivo* population label yield a similar linear plot, but in this case the "tail" presumably is due to recycling of the isotope.<sup>41,233</sup> When  $^{51}\text{Cr}$ -chromate was used as a cohort label in rats,<sup>143</sup> less than one third of the platelet loss appeared to be due to random destruction, a conclusion confirmed by recovery of the majority of the isotope from the reticuloendothelial system.<sup>14</sup>

On the other hand, survival curves obtained in animals with  $\text{DF}^{32}\text{P}$ -labeled platelets were curvilinear,<sup>121</sup> and the survival of cohorts labeled with  $^{35}\text{S}$ -sulfate and  $^{75}\text{Se}$ -selenomethionine appeared to be determined mainly by random loss. Similar results were obtained with platelets labeled with both  $^{35}\text{S}$  and  $\text{DF}^{32}\text{P}$ ,<sup>257</sup> a technique that provides a combined population and cohort label. The possibility remains that many of these discrepancies represent valid species differences.

The vast majority of available data suggests that platelet survival curves in man are neither truly rectilinear nor first-order,<sup>172</sup> and that both random destruction and senescence determine the fate of the platelet.<sup>172</sup> Many have concluded that senescence predominates under normal circumstances,<sup>16,83,288,332</sup> while others maintain that the major factor is random loss,<sup>96,116,305</sup> or favor more complicated models.<sup>286</sup> This question cannot be answered with available data and must await the development of new or improved labeling techniques.

### Ineffective Thrombopoiesis

A direct and linear relationship between platelet turnover rate, as measured by isotopic techniques discussed above, and the total megakaryocyte mass has been demonstrated in man<sup>161</sup> and in rats<sup>158</sup> (Fig. 9-5).

This relationship also holds true in a wide variety of disorders characterized by thrombocytopenia or by "reactive" thrombocytosis (Chapter 36). However, in thrombocytopenia associated with megaloblastic anemias (page 1097) and certain other disorders, this correlation is not seen (Fig. 9-5). The latter suggests that thrombopoiesis is "ineffective" in these disorders,<sup>161</sup> a phenomenon discussed in detail in Chapter 34.

### Regulatory Processes

The remarkable constancy of the platelet count under normal circumstances suggests that the production of these cells is well regulated, and the presence of humoral controlling factors analogous to erythropoietin (Chapter 4) has been postulated for many years.<sup>2,84</sup> Studies demonstrating the enhancement of platelet production by thrombocytapheresis and its depression by platelet transfusion provide good evidence for the presence of such a "thrombopoietin."<sup>326,377</sup> In animals, the effects of this humoral substance may be roughly quantified by measurements of the rate of RNA synthesis, and by determination of total platelet production rate by means of cohort labeling.<sup>126,159,340</sup> Several groups of workers have demonstrated stimulation of thrombopoiesis in animals given serum, plasma and fractions thereof, and urine derived from human and animal sources.<sup>223,238,324</sup> The results of such studies have not been entirely consistent,<sup>2</sup> and numerous nonspecific factors tend to confuse the results.<sup>324</sup> Newly developed assay systems may provide more adequate methods of studying "thrombopoietins."<sup>120,341</sup>

Attempts to isolate and chemically characterize thrombopoietic substances have met with only limited success. Preliminary studies suggest that an active principle migrates electrophoretically as an alpha-2 globulin, contains carbohydrate, is not dialyzable, and is thermostable.<sup>369</sup> In these respects it resembles erythropoietin, but it differs from this substance in terms of storage lability, its presence in nephrectomized animals,<sup>106</sup> and in several other respects.<sup>106,270,369</sup>

There is some evidence that a humoral thrombopoietic factor acts on the stem cell compartment.<sup>326</sup> Data obtained by means of the spleen-colonization technique in lethally irradiated mice (page 50) suggest that transplanted cells, possibly the megakaryocyte-committed stem cells, retain "thrombopoietic direction."<sup>122</sup>

Studies of cyclic variations in the platelet count in normal individuals,<sup>332</sup> and in patients with cyclic thrombocytopenia and tidal platelet dysgenesis (page 1098), suggest that the control of thrombopoiesis involves a negative "feedback" mechanism that contains a time delay. As a result of this time lag,

"perturbations" of either a positive or negative nature may be magnified, resulting in an oscillation of platelet production. More than one humoral regulator, or a combination of negative and positive "feedback" regulatory processes, may be involved.<sup>118,160,172,194</sup> Claims for the presence of thrombopoietic substances or inhibitors in the spleen<sup>82,310,404</sup> are not convincing.<sup>2,84,322</sup>

Clinical evidence for the presence of a humoral "thrombopoietin" is provided by a remarkable patient with a chronic, presumably congenital form of thrombocytopenia.<sup>369</sup> In this patient, who has been studied for over 15 years, the transfusion of normal plasma

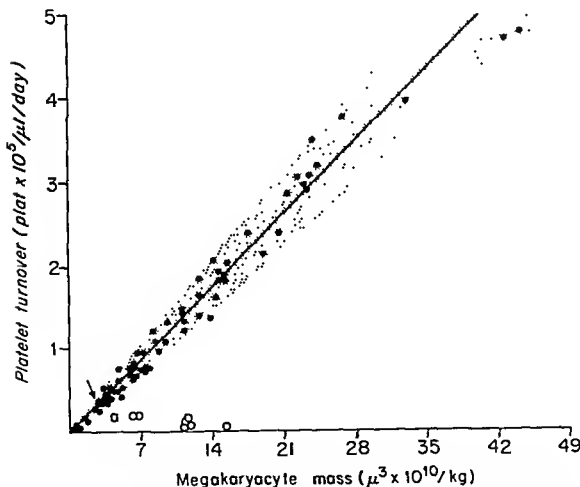


Fig 9 5. Correlation between platelet turnover and megakaryocyte mass. The two measurements of platelet production were compared by plotting megakaryocyte mass against platelet turnover. The correlation indicates that these are equivalent measurements. The normal mean value  $\pm 1$  SD is represented by the black square located by the arrow. The confidence limits ( $\pm 1$  SD) are shown in the shaded area. In eight patients (open circles), marked disparity was seen between platelet turnover and megakaryocyte mass, a finding indicating significant ineffective thrombopoiesis (From Harker and Finch,<sup>161</sup> courtesy of the authors and the Journal of Clinical Investigation)

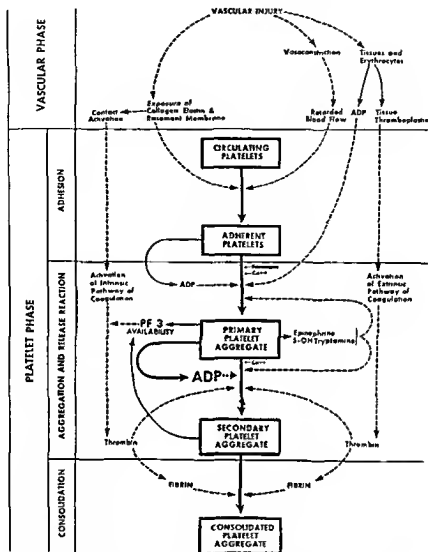


Fig 9-6 The vascular and platelet phases of hemostasis (primary hemostasis). Action is denoted by dashed lines, transformation including for purposes of illustration the release or activation of substances contained within the platelet, is illustrated by solid lines

(page 423) and platelet adhesion, discussed below, both of which are initiated by the exposure of collagen, and the initiation of the extrinsic pathway of coagulation by thromboplastins released from injured tissues (Chapter 10).

## The Platelet Phase of Hemostasis

In mammals, the major function of the platelet is hemostasis,<sup>53,244</sup> a process in which

this cell plays both a mechanical and a biochemical role. Hemostasis probably proceeds in the essentially stepwise manner indicated in Figure 9-6. Vascular injury results first in the adhesion of platelets to the vessel wall. Such adherent platelets release stored ADP (the release reaction). This initiates the formation of aggregates between platelets. The activation or "availability" of platelet factor 3 and the initiation of blood coagulation then follow, leading to the consolidation of the platelet plug by fibrin and subsequently to the phenomenon of clot retraction.

Platelet functions that are unrelated to hemostasis are discussed briefly on page 399. Numerous excellent reviews and monographs provide further details concerning platelet function.<sup>53,100,144,172,242,249,251,269,290,292,317</sup>

### Platelet Adhesion

The phenomenon of *platelet adhesion* (the attachment of platelets to non-platelet surfaces<sup>394</sup>) can be dissociated from *platelet aggregation* (the attachment of platelets to one another). In the presence of EDTA, a chelating agent that inhibits platelet aggregation, individual platelets adhere in a single layer to injured vessel segments without forming aggregates.<sup>393,395</sup> When ADP is infused into animals, platelet aggregates form in the blood stream but do not adhere to vascular surfaces.<sup>381</sup>

In experimental wounds, platelets appear to adhere mainly to exposed *collagen fibers* (Fig. 9-6).<sup>192,375</sup> This involves a specific biochemical interaction, which may be the formation of an enzyme-acceptor complex between incomplete heterosaccharide-lysine groups of collagen<sup>220a</sup> and glycosyl transferases in the platelet membrane.<sup>202</sup> Neuraminidase in the platelet membrane may expose acceptor groups for this enzyme by hydrolyzing terminal sialic acid residues in collagen.<sup>58</sup> This hypothesis is consistent with data demonstrating that the activity of collagen depends on its native fibrillar structure,<sup>168a</sup> intact galactosyl residues,<sup>262</sup> free sulfhydryl groups,<sup>10</sup> and the presence of epsilon amino groups of lysine,<sup>439</sup> but is unaffected by removal of the telopeptides or the blocking of free carboxyl groups.<sup>442</sup> The endothelial basement membrane also serves as a site of platelet adhesion,<sup>27,197,412</sup> and, since it contains acceptor groups similar to those of collagen, may interact with the platelet by means of a similar biochemical mechanism.<sup>202</sup> In large vessels which lack a basement membrane, platelets appear to adhere to *elastic fibers*.<sup>394</sup> Data concerning the role of the endothelial cell per se in platelet adhesion are contradictory.<sup>53,136,242,394</sup>

The adhesion of platelets to injured vascular surfaces also is conditioned by the ad-

sorbed protein coating of both the vessels and the platelets,<sup>362,394</sup> although the importance of specific plasma protein cofactors in this process has not been established. The ability of collagen to induce platelet aggregation is diminished as the result of interaction with certain normal plasma proteins.<sup>313</sup> Poorly understood rheologic factors also are probably of great importance in platelet adhesion, eg, the concentration of other cells in the perfusing blood, the rate of blood flow, the velocity gradient.<sup>26,93,167,234</sup>

Platelet adhesion to collagen and other biologic surfaces should not be confused with adhesion of platelets to glass or other foreign surfaces (page 1054). The latter may reflect quite different phenomena,<sup>362</sup> eg, the effects of the "anti VW factor" (page 1182), ADP-induced aggregation.

### Platelet Aggregation

Platelet aggregation follows adhesion of platelets to injured surfaces, and normally is apparent in experimental wounds within 15 seconds.<sup>192</sup> ADP is the central factor in this process (Fig. 9-6). Most evidence favors the view that ADP derived from injured tissues and erythrocytes<sup>339</sup> is of minor importance, and that, in a wound, aggregation is initiated mainly by ADP extruded from adherent platelets themselves during the release reaction.<sup>194,290</sup>

### Adenosine Diphosphate

Platelet aggregation induced by very low concentrations of ADP (0.1 to 0.4  $\mu\text{M}$ ) ("primary" aggregation) is reversible as regards both morphologic and biochemical changes.<sup>155</sup> At intermediate concentrations (0.4 to 1.5  $\mu\text{M}$ ) ("threshold concentrations"), aggregometer tracings reveal an initial "primary" wave of reversible aggregation followed by a second wave of irreversible aggregation that is produced by ADP released from the primary platelet aggregate ("secondary" aggregation) (Fig. 9-7).<sup>155,245</sup> With higher concentrations of ADP, the primary and secondary waves merge. In a minority of normal subjects biphasic aggregation does

## Miscellaneous Aggregating Agents

In vitro, platelet aggregation can be initiated by a variety of substances, all of which presumably act by inducing the release of ADP from the platelet. These include free fatty acids,<sup>163,177</sup> immunoglobulins<sup>101,343a</sup> and antigen-antibody complexes,<sup>283</sup> unconjugated bilirubin,<sup>260</sup> viruses<sup>206</sup> and bacterial toxins,<sup>189,205</sup> vasopressin,<sup>165</sup> snake venoms,<sup>101</sup> polymerizing fibrin monomers,<sup>390</sup> phytohemagglutinins,<sup>216a</sup> and even ultraviolet light.<sup>111</sup> Aggregation and the release reaction also may be initiated by many foreign particles and surfaces,<sup>89</sup> the effects of which are greatly modified by coating with various proteins.<sup>330</sup>

## The Platelet Release Reaction

A number of substances that are contained principally in the storage organelles and lysosomes of the platelet are extruded from the cell when it is physiologically stimulated.<sup>149,229</sup> This occurs rapidly,<sup>445</sup> involves the expenditure of considerable energy, and does not affect cytoplasmic or mitochondrial enzymes<sup>53</sup> or the integrity of the platelet membrane.<sup>179</sup> This phenomenon has been termed the platelet release reaction (Fig. 9-6), and is quite comparable to specific secretory functions of other cells.<sup>104,149,403</sup>

A remarkable diversity of biologically active substances are extruded from the platelet during the release reaction.<sup>172,307,455</sup> In addition to those already mentioned, these include calcium ions,<sup>415</sup> fibrinogen,<sup>149,222</sup> ATP, various vasoactive substances,<sup>303</sup> inorganic pyrophosphate,<sup>380a</sup> potassium ions,<sup>50,154</sup> various enzymes including acid phosphatase and beta glucuronidase,<sup>457</sup> platelet factor 4 (page 398),<sup>309,407</sup> and an "elastolytic" protease.<sup>453</sup> In view of the known biologic activity of many of these substances, it has been assumed that their release by the platelet has some physiologic significance, but there is little direct evidence for this hypothesis. There is some evidence that vasoactive substances released from the platelet may be of pathophysiologic importance in thromboembolic

disorders (Chapter 39) and in intravascular coagulation (Chapter 38). Permeability factors released from the platelet may have a physiologic role in inflammation<sup>294</sup> and possibly a pathologic role in inflammatory disorders such as rheumatoid arthritis.<sup>88</sup>

Large amounts of beta glycerol acid phosphatase are released into the plasma when platelets are damaged in vivo by various pathologic processes.<sup>332</sup> Measurements of plasma levels of this enzyme may provide a means of differentiating between thrombocytopenia caused by increased platelet destruction, and that resulting from deficient production (Chapter 34).

## The Role of ADP

ADP apparently is central to the hemostatic function of the release reaction. Owing to the exquisite sensitivity of the platelets to this nucleotide, even the small amounts of ADP released from adherent platelets are sufficient to induce aggregation of a much larger number. The resulting aggregate in turn releases ADP, leading to a still larger aggregate. It is thus difficult to delineate the phenomena of platelet adhesion, aggregation, and the release reaction with respect to time. At some point, the process becomes irreversible, a feature corresponding to the in vitro phenomenon of "secondary" aggregation discussed above.<sup>242</sup> The release reaction may be viewed as a "biologic amplifier" that rapidly converts a minimal stimulus into a massive hemostatically effective response.<sup>307</sup>

The ADP extruded during the release reaction originates in the storage nucleotide pool located within the platelet dense bodies. Approximately 50% of the storage ADP is released, this averaging 1.6  $\mu\text{M}/10^8$  platelets.<sup>455</sup> The energy required to operate the release mechanism is not derived from the storage pool, but rather from the breakdown of ATP from the metabolic nucleotide pool (Fig. 9-2).

The biochemical mechanism that initiates the release reaction is poorly understood. The substances extruded, as well as the concomitant "burst" of metabolic activity, vary



both qualitatively and quantitatively, depending on the agent that induces the release reaction.<sup>138,231,292,419,457,458</sup> The process is facilitated by calcium<sup>191</sup> but is not strictly dependent on this ion,<sup>389</sup> and may require a plasma cofactor distinct from plasma fibrinogen.<sup>90</sup> The release reaction induced by zymosan, inulin, and bacterial endotoxin depends on a preliminary interaction with the sixth component of complement (C' 6) in the plasma.<sup>382</sup> Under physiologic conditions, the major initiating factor may be close contact with "active" surfaces and other platelets.<sup>257</sup> The normally concurrent phenomena of aggregation and platelet factor 3 availability apparently are not essential for the release reaction, which appears to be a biochemically discrete event.

#### The Role of Cyclic AMP and Prostaglandins

The level of cyclic AMP (cAMP) in "resting" platelets is approximately 140 pM/10<sup>9</sup> platelets.<sup>365</sup> Despite active turnover, this low level is normally maintained within narrow limits by the action of two enzymes (Fig. 9-8), namely *adenyl cyclase*, which catalyzes the synthesis of cAMP from ATP,<sup>444</sup>

and *phosphodiesterase*, which degrades cAMP to AMP. Cyclic AMP inhibits ADP-induced platelet aggregation in vitro, but only in high concentrations,<sup>256</sup> a fact attributable to its rapid degradation by phosphodiesterase and its inability to penetrate the platelet membrane.<sup>348</sup> A congener, dibutyl cAMP, penetrates the cell rapidly, is not destroyed by phosphodiesterase, and is a potent inhibitor of platelet aggregation.<sup>256</sup>

*Inhibitors of platelet aggregation* increase the levels of cAMP in the platelet. Many of these act as inhibitors of phosphodiesterase, eg, various methyl xanthines,<sup>451</sup> papaverine, possibly adenosine and 2-chloroadenosine.<sup>185,273</sup> Prostaglandin E<sub>1</sub> (PGE<sub>1</sub>) in low concentrations (1 μM) stimulates the action of adeny cyclase.<sup>444,451</sup> This substance inhibits both primary and secondary ADP-induced aggregation and the release reaction induced by thrombin, epinephrine, and other agents.<sup>224,374,445</sup> Other prostaglandins are active but are much less potent.<sup>227,451</sup>

Evidence concerning the relationship between cAMP and platelet aggregating agents is much less clear. Most substances that initiate platelet aggregation decrease platelet levels of cAMP. Epinephrine,<sup>267</sup> 5-HT, and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) may act as inhibi-

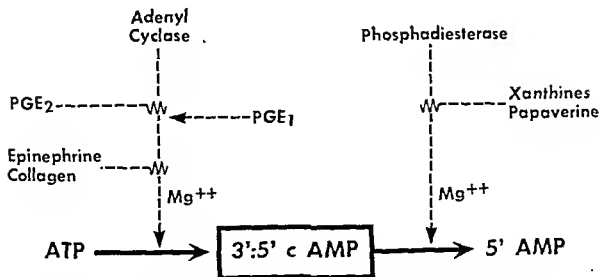


Fig 9-8 Agents that affect platelet levels of cyclic AMP. Dashed arrows indicate action, solid arrows indicate transformation.

tors of adenylyl cyclase<sup>274,415,451</sup>; collagen fibers and various foreign particles produce a similar effect.<sup>365</sup> Prostaglandins  $E_2$  and  $F_{2\alpha}$  apparently are synthesized in the platelet.<sup>388a</sup> They are released when the platelet is "stimulated," eg, by thrombin.<sup>37</sup> These data suggest that  $PGE_2$  may act as a physiologic counterbalance to the effects of  $PGE_1$ . ADP leads to a reduction in platelet cAMP levels, but has no effect on the activity of adenylyl cyclase or phosphodiesterase in cell homogenates.<sup>274,365</sup> Contradictory results have been obtained with thrombin. In some studies, this enzyme reduced cAMP levels and inhibited adenylyl cyclase<sup>365</sup>; in others, an increase in cAMP was observed.<sup>168</sup>

The data discussed above provide substantial evidence that cAMP and certain prostaglandins play an important role in mediating the responses of the platelet to various stimuli.<sup>227</sup> However, factors other than those involving adenylyl cyclase and phosphodiesterase may be of equal importance in regulating the levels of cAMP in the platelets.<sup>374</sup>

### Morphologic Changes

Most evidence would suggest that adhesion alone does not produce significant alterations in the form and structure of the platelets. The first definite change seen in vivo, or when ADP is added to platelets in vitro, is a rapid transformation of normally discoid platelets into spheres with numerous small protrusions or pseudopods ("spiny spheres").<sup>52,193</sup> This *initial shape change* is seen just before or at the same time as the release reaction. It occurs in the absence of calcium ions and thus is apparent even when aggregation is inhibited by EDTA or is lacking altogether, eg, in platelets from patients with thrombasthenia.<sup>460</sup> Electron photomicrographs reveal that the shape change is associated with a gathering together of various organelles in the center of the cell. This has been ascribed to the contraction of the marginal microtubules,<sup>429</sup> which may undergo a transformation into fibrils.<sup>65,428</sup> Data obtained by electronic particle counters which suggest that the initial shape change is accompanied by an increase in platelet volume<sup>65</sup> have been disputed.<sup>52</sup>

It has been suggested that the shape change may expose reactive groups on the platelet surface, or that the formation of pseudopods may act to stabilize platelet aggregates<sup>193</sup> and thus facilitate intimate adhesion of platelets to tissues and other cells.<sup>53</sup> There is no direct evidence for any of these hypotheses.

Chilling<sup>30,220,428</sup> and prolonged incubation with EDTA or cocaine<sup>65,364</sup> induce platelet "swelling." This phenomenon differs fundamentally from the shape change, and presumably is the result of the impairment of metabolic processes required to maintain a normal cell configuration; it may reflect reversible depolymerization of the microtubules.<sup>28,30,256,364</sup>

Platelets in aggregates formed in vitro by sub-threshold concentrations of ADP ("primary" aggregates) reveal only the shape change, a morphologic change that disappears as deaggregation occurs.<sup>155</sup> With higher concentrations of ADP ("secondary" aggregates), platelet pseudopods become longer, but individual platelets remain distinct and do not fuse.<sup>57,149,155,242</sup> However, slight shrinkage ("cohesion") of the aggregate may be seen. These morphologic changes are most marked in platelets in the superficial layers of the aggregate, and in those adherent to the endothelium,<sup>155,192</sup> and correspond to those commonly referred to by the term *viscous metamorphosis*. Many observers have reported platelet degranulation in secondary aggregates, but this finding has not been observed consistently.<sup>155,422</sup>

The structure of platelet plugs formed in vivo, although quite similar to the above, is more variable because of rheologic factors. The platelet plug adheres to adjacent morphologically normal endothelium,<sup>373</sup> and may either occlude or cap the end of the injured vessel. Although still permeable to some red cells, platelet plugs alone, without consolidating fibrin, may be hemostatically effective in small vessels.

### The Role of Platelets in Blood Coagulation

Various protein or lipoprotein substances derived from the platelet have come to be

designated as platelet "factors" because of their apparent function in blood coagulation. These are designated by Arabic numerals, in distinction to the Roman numerals used in referring to the coagulation factors (Table 10-1, page 410).

### Platelet Factor 3

Platelet factor 3 (PF-3) is required in at least two steps in the process of blood coagulation, namely, the interaction between factors IXa and VIII which results in the activation of factor X (Fig. 10-4, *reaction 4*, page 424), and the interaction between factor Xa and factor V which leads to the formation of prothrombinase (*reaction 7*). Platelet factor 3, or a substitute phospholipid, also is required for the activation of factor X by the venom of Russell's viper (*Stypven*) (*reaction 14*). Details of these reactions are discussed in the following chapter.

Platelet factor 3 is a thermostable lipoprotein. When isolated from platelet homogenates, it is found mainly in the particulate fractions that contain platelet membranes and granules<sup>292</sup>; it also is found in platelet "dust."<sup>443</sup> When "activated" by ADP and other substances under various *in vitro* conditions, PF-3 remains closely associated with the platelet membrane.<sup>154,172,188</sup>

Non-sedimentable material with PF-3 activity can be demonstrated in serum,<sup>315</sup> and following protracted incubation of platelets with thrombin.<sup>101,187</sup> The significance of such "soluble" PF-3 is difficult to assess, since assays for PF-3 activity are quite non-specific. Phospholipids which substitute for platelets in all of the various assay methods can be extracted from most tissues, including erythrocytes.<sup>75</sup> Indeed, a variety of substances ranging from crude fractions of cephalin or soybean phosphatides to purified phosphatides exhibit a comparable activity. None of these PF-3 "substitutes" is as potent as isolated platelet membranes.<sup>252</sup> It has been suggested that "soluble" PF-3 plays no physiologic role under normal circumstances and represents solubilized membrane lipoprotein or phospholipids which passively leak from dead cells.<sup>172,219</sup> It is for this reason that

PF-3 activity which is demonstrated in frozen or sonicated whole platelet suspensions, although often assumed to represent a quantitative measure of "total PF-3," has little specific significance.

The evidence cited above would suggest that PF-3 is in reality the platelet membrane which, when suitably "activated" or "made available," acquires the ability to bind and orient activated coagulation factors. The complex so formed acquires enzymatic properties, i.e., the ability to activate either factor X or prothrombin.

The precise interrelationships between platelet aggregation, the release reaction, and PF-3 availability remain uncertain.<sup>419</sup> The three phenomena normally are closely related, and the amount of PF-3 made available during ADP-induced aggregation is roughly proportional to the extent and duration of aggregation.<sup>154,385</sup> There is, however, evidence that neither ADP nor the release reaction is essential.<sup>18,138,317</sup> Platelet factor 3 becomes available after the release of ADP and 5-HT<sup>385</sup> and before thrombin formation or visible alterations in the platelet membrane are apparent<sup>219</sup>; under some experimental circumstances it may even be a reversible phenomenon.<sup>317</sup> Most data favor the hypothesis that PF-3 availability, like the release reaction, is initiated as the result of close apposition of the platelet to "active" surfaces,<sup>392</sup> including other platelets.<sup>138</sup>

Under certain *in vitro* conditions, platelets acquire an activity similar to that of a complete tissue thromboplastin; i.e., they are capable of activating factor VII and the extrinsic pathway of coagulation<sup>10</sup> (Chapter 10). This phenomenon appears to depend on contact activation and factor XII. There is indirect evidence that platelets also may initiate the process of contact activation.<sup>70,424</sup> Studies suggesting that this is mediated by ADP released from the platelets require confirmation.<sup>315</sup> None of these reactions is of established physiologic significance.

### Other Platelet Factors

*Platelet factor 2* (PF-2) (fibrinogen activating factor) is a globulin of indeterminate

molecular weight. When semipurified fractions of this substance are incubated with fibrinogen, nonprotein nitrogen is released, suggesting a proteolytic action. In vitro, PF-2 inhibits antithrombin III, induces platelet aggregation, and accelerates the rate of the thrombin-fibrinogen reaction.<sup>127,308</sup>

*Platelet factor 4* (PF-4) (antiheparin factor) is a small glycoprotein or large glycopeptide of great thermostability and unusual resistance to proteolysis. This substance is extruded together with ADP during the release reaction,<sup>306</sup> although under certain in vitro conditions its activity remains localized to the platelet membrane.<sup>317,318</sup> In vitro, PF-4 facilitates ADP-induced aggregation under certain circumstances,<sup>241</sup> and antagonizes the anticoagulant effects of heparin and those of certain fibrinogen degradation products (page 436). It precipitates soluble fibrin monomers, and in high concentrations may also precipitate fibrinogen.<sup>241,309</sup> These properties may play a role in the pathophysiology of intravascular coagulation and in various "paracoagulation" phenomena (page 1217).<sup>241</sup>

The term "platelet factor 1" refers to adsorbed plasma coagulation factor V<sup>176</sup> and platelet factor 5 refers to platelet fibrinogen (page 378). A plasmin inhibitor associated with the platelets is sometimes termed "platelet factor 6."<sup>124</sup> The nature and physiologic importance of platelet factor 7 ("co-thromboplastin"), PF-8 (antithromboplastin), and PF-9 (accelerator globulin stabilizing factor) are obscure. These terms, and platelet factor 10 (5-hydroxytryptamine), are rarely used.

### The Consolidation Phase

Fibrin acts to reinforce the primary or temporary platelet thrombus,<sup>374a</sup> and usually is apparent in experimental hemostatic plugs within one minute or even earlier. Fibrin strands are first seen on the surface and in the inner layers of the thrombus, particularly in association with those platelets that are adherent to the endothelium.<sup>8,192</sup> Fibrin often

is absent among the intervening layers of platelets.<sup>221,375</sup> In the presence of continued blood flow, fibrin is deposited as a cap over the entire thrombus and adjacent normal endothelium. The end result of these events, which are arbitrarily termed the "consolidation phase," is the permanent hemostatic plug that has become impermeable, more dense, mechanically stronger, and slightly diminished in volume.<sup>194</sup>

In common with the formation of the primary platelet thrombus, the events of the consolidation phase vary depending on rheologic factors.<sup>375</sup>

### Clot Retraction

There is little evidence in support of older theories that attributed the phenomenon of clot retraction to nonspecific syneresis of fibrin or mechanisms extrinsic to the platelet acting directly on fibrin.<sup>39</sup> In whole blood, clot retraction usually is slow and is deficient if the platelet count is below  $50 \times 10^9/\mu\text{l}$ . In platelet-rich plasma, retraction is more rapid, and in extent is directly proportional to the platelet count, if it is less than  $100 \times 10^9/\mu\text{l}$ , and is inversely proportional to the fibrinogen concentration.<sup>39,64</sup>

Clot retraction apparently begins when platelet clumps adhere to fibrin strands. This occurs at an undefinable point in the consolidation phase, and in a relatively specific manner; ie, the platelets adhere at the point where fibrin strands cross one another ("nodes"), and their pseudopods attach to the radiating fibrin strands.<sup>32,192</sup> This is thought to produce a three-dimensional lattice<sup>409</sup> such that, when the pseudopods shorten, contraction of the entire mass occurs. In platelet-rich plasma, this may produce a reduction of as much as 90% in the volume of the clot.<sup>39</sup> The adhesion of platelets to fibrin is an essential preliminary to clot retraction, but the process is poorly understood. There is some evidence that it is mediated by the interaction of platelet fibrinogen with fibrin strands of plasma origin,<sup>125,436</sup> and that it may involve PF-4.<sup>309</sup> Stabilized fibrin is less readily retracted than is non-stabilized fibrin.<sup>311</sup>

The mechanical work of clot retraction is carried out by thrombosthenin (page 379),<sup>34,302</sup> acting as a  $Mg^{++}/Ca^{++}$ -dependent adenosine triphosphatase.<sup>302</sup> Glucose is utilized and ATP is synthesized throughout the entire process. The complete abolition of clot retraction by metabolic inhibitors requires inhibition of both glycolytic and oxidative pathways; either pathway alone is able to provide sufficient energy for the process.<sup>112,284</sup> Thrombin and calcium ions also are required for clot retraction.

Clots formed by the enzyme reptilase do not retract.<sup>282</sup> It is uncertain whether this is due to a specific effect of this enzyme on the platelet<sup>310</sup> or the fact that reptilase removes only fibrinopeptide B from fibrinogen (page 412).<sup>282</sup>

The *homeostatic significance* of clot retraction has been debated extensively and remains unsettled. The phenomenon has been likened to a physiologic "ligature"<sup>134,135</sup> that progressively pulls the sides of an injured vessel together. The extensive studies of Budtz-Olsen<sup>64</sup> would suggest, to the contrary, that the mechanical force of clot retraction, estimated to be less than 20 mm of water in whole blood, is too feeble to produce such an effect. Budtz-Olsen suggested that clot retraction is a vestigial hemostatic function. Clot retraction may, however, exert a significant mechanical force in hemostatic plugs composed mainly of platelets, in contrast to whole blood clots, where the process is impeded by trapped erythrocytes.<sup>39</sup>

## Homeostatic Control Mechanisms

Several processes that could act to limit the intravascular propagation of the platelet thrombus have been demonstrated *in vitro*, but their homeostatic importance is uncertain.<sup>172</sup> Thus, the plasma contains adenylyl kinase, an enzyme that rapidly breaks down free ADP.<sup>182</sup> Electron microscopic studies would suggest that the superficial layers of the primary hemostatic plug are composed of reversibly aggregated or "refractory" platelets,<sup>360</sup> which may deaggregate and be

washed away. Evidence that ATP, adenosine formed in the plasma from ATP,<sup>360</sup> and serotonin<sup>24</sup> inhibit ADP suggests that the release of these substances in high concentrations may constitute a physiologic inhibitor of further platelet aggregation.<sup>25</sup> There is preliminary evidence that platelet deaggregation is specifically induced by stimulation of beta adrenergic receptors on the platelet,<sup>1a,63</sup> a phenomenon that may be mediated by cAMP. When consolidation of the primary hemostatic plug occurs, the platelet aggregate presumably is excluded from the circulation by a fibrin barrier, which may limit the size of the platelet thrombus.

## Miscellaneous Platelet Functions

Various properties of the platelet that are not concerned primarily with hemostasis will be discussed in this section. The evidence for the physiologic importance of these "functions" is incomplete, and various reviews of these specialized topics should be consulted for more details.<sup>283,201,292,294,298</sup> The role of platelets in fibrinolysis is discussed in Chapter 10.

### Endothelial Support

A long-standing hypothesis holds that *platelets act to maintain or support the integrity of the vasculature by attaching to gaps which normally develop in the endothelium, or as the result of their actual entrance and incorporation into endothelial cells.*<sup>208,209,417,441</sup> When isotopically labeled platelets are infused into thrombocytopenic animals, radioactivity may be subsequently demonstrated in the endothelium.<sup>91,411</sup> However, this phenomenon could not be demonstrated in normal animals.<sup>92</sup> Certain electron photomicrographs have been interpreted as showing the actual incorporation of platelets into endothelial cells,<sup>208</sup> but numerous ultrastructural studies of a similar nature have failed to reveal this phenomenon. The results of such studies are difficult to evaluate in any case,<sup>251</sup> and there is a large body of data

suggesting that platelets do not adhere to entirely normal endothelium.<sup>175,251</sup> Radiation-induced thrombocytopenia results in the rapid appearance of erythrocytes in the lymph of dogs,<sup>417,446</sup> but neither vascular fragility nor uncomplicated thrombocytopenia in man is necessarily associated with increased vascular permeability, as measured by the loss of <sup>131</sup>I-labeled albumin.<sup>421</sup> In vitro perfusion of the thyroid with platelet-rich plasma prevents degenerative changes that develop in the endothelium when platelet-poor plasma is used.<sup>142</sup> This effect could not be confirmed with perfused kidneys.<sup>427</sup> Finally, studies of platelet survival and sequestration in man, as discussed on page 386, would suggest that only a small proportion of platelets is consumed by random processes under normal circumstances; this is not significantly increased in patients with protracted thrombocytopenia caused by deficient platelet production.<sup>161</sup>

The available evidence favors the conclusion that platelets are utilized to *repair* small or imperceptible vascular injuries rather than to *support* or *maintain* normal endothelium.

## Phagocytosis

There is evidence that platelets, by means of a process similar to phagocytosis, engulf various foreign particles, eg, Thorotrast, India ink, carbon, polystyrene, and some bacteria.<sup>130,291</sup> It is often difficult to differentiate between true phagocytosis and passive attachment of particles to the membrane which lines the surface connecting canalicular system<sup>86,432</sup> or entrapment of particulate matter in the canalicular system during fixation. For example, myxoviruses are attached to the platelet surface coat, including that which lines the canalicular system, by means of specific glycoprotein receptors.<sup>206</sup>

The physiologic significance of platelet "phagocytosis" is obscure. It plays no apparent role in defense against infection, but may provide an ancillary, possibly vestigial mechanism for clearing particulate material from the blood.<sup>416</sup>

## Transport Functions

The platelet concentrates various substances by means of active metabolic processes. These active "transport" functions should be distinguished from passive adsorption;<sup>172,209</sup> which was discussed earlier (page 378). The platelets contain virtually all of the blood 5-hydroxytryptamine (5-HT), which is concentrated by means of a complicated biochemical process. Epinephrine<sup>56,57</sup> and potassium also are concentrated by means of active processes,<sup>81</sup> the latter presumably involving the same membrane receptor as 5-HT.<sup>24</sup> The release of potassium from platelets during coagulation is a common cause of "pseudohyperkalemia" (page 1106).

The assumption that the active and specific transport of such biologically potent substances by the platelet serves a purpose would seem reasonable, but supporting evidence is scanty. Abnormalities in platelet transport of 5-HT have been demonstrated in multiple sclerosis,<sup>68</sup> Down's syndrome,<sup>59</sup> and in certain other disorders.<sup>63</sup>

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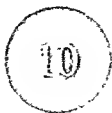
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## Blood Coagulation

- The Coagulation Factors
  - Fibrinogen
  - The Vitamin K-Dependent Factors (Prothrombin, Factors VII, IX, X)
  - Factor V
  - Factor VIII
  - Factor XI
  - Factor XII
  - Factor XIII
  - Tissue Factor
  - Miscellaneous Coagulation Factors
  - Variations in Health and Disease
- The Physiology of Coagulation
  - The Intrinsic Pathway
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  - The Common Pathway
  - Miscellaneous Coagulation Phenomena
- Homeostatic Control Mechanisms
  - Local Processes
  - Humoral Inhibitors
  - Cellular Clearance Mechanisms
- The Fibrinolytic Enzyme System
  - Components of the System
  - The Physiology of Fibrinolysis
  - The Proteolytic Degradation of Fibrin;  
Degradation Products
- Homeostatic Significance of Coagulation
  - Hemostasis
  - Non-hemostatic Processes

of a visible coagulum, which is the physical manifestation of fibrin formation, represents only the end result of an intricate series of reactions that involve a number of factors. Despite rapid advances in the past 30 years, many aspects of the process of blood coagulation still are poorly understood, and this field remains one of the major "frontiers" of hematologic research.

For more detailed information than can be included in the following summation, the reader is referred to the many excellent books, monographs, and reviews concerned generally with this topic,<sup>59,62,145,225,365,381,450</sup> and to those that deal with its various special aspects, eg, immunology,<sup>110</sup> kinetics,<sup>59,140,195,196</sup> comparative physiology.<sup>5,120,191,302,305</sup> Much information concerning normal coagulation has been obtained from the study of patients and animals with hereditary deficiencies of the various coagulation factors; this is summarized in Chapter 37. Assays for the various coagulation factors are discussed in Chapters 33 and 37. The role of coagulation factors associated with platelets was discussed in Chapter 9.

### The Blood Coagulation Factors

#### Nomenclature

The nomenclature of the coagulation factors has been notoriously inconsistent and

**B**LOOD coagulation was one of the first biologic processes to be studied experimentally, and many workers better known for their contributions to other disciplines were intrigued by the phenomenon, including Hunter, Lister, Virchow, Arthus, and Bordet.<sup>304</sup> The apparent simplicity of coagulation proved to be deceptive. The formation

confusing. Substances involved in this process which were defined prior to the present century received descriptive names, eg, fibrinogen, thrombin, prothrombin, thromboplastin. As information accumulated, new factors were named according to elementary biochemical or functional properties, eg, "labile" factor, "serum prothrombin conversion accelerator." More recently, they have received the surnames of the kindreds in whom hereditary deficiencies of the factors were first discovered, eg, Christmas, Stuart, Hageman. In the resulting confusion, several different terms for the same substance often were in simultaneous use.

These problems have been partially solved by the development of an international standard nomenclature.<sup>5,13</sup> Each coagulation factor is designated by a Roman numeral (Table 10-1). The term "factor VI," which originally referred to the activated form of factor V, has been abandoned. The descriptive terms "fibrinogen," "prothrombin," "calcium," and "tissue factor" will be used

here in preference to the Roman numerals indicated in Table 10-1.

The international nomenclature clearly distinguishes between the coagulation factors and the numbered factors related to the platelets; the latter are designated with Arabic numerals (page 397). It also can be adapted to a "shorthand" representation of coagulation reactions. By convention, activated forms of factors are designated by adding "a." For example, the conversion of factor X into its active form is written:  $X \rightarrow X_a$ .

### General Properties

Some elementary properties of the coagulation factors are summarized in Table 10-2. It should be emphasized that much of the basic information concerning these proteins is of limited accuracy. With the exception of fibrinogen and prothrombin, the coagulation factors are trace proteins, and in most instances information concerning their biochemistry (column 2) has been obtained from

Table 10-1. Nomenclature and Synonyms for Coagulation Factors

Roman Numeral	Preferred Descriptive Name	Synonyms
✓ I	Fibrinogen	
✓ II	Prothrombin	
✓ III	Tissue factor	Thromboplastin
✓ IV	Calcium ions	
I V	Proaccelerin	Labile factor, accelerator globulin (AcG), thrombogen
✓ VII	Proconvertin	Stable factor, serum prothrombin conversion accelerator (SPCA), autoprothrombin I, cothromboplastin
✓ VIII	Antihemophilic factor (AHF)	Antihemophilic globulin (AHG), antihemophilic factor A, platelet cofactor 1, thromboplastinogen
✓ IX	Plasma thromboplastin component (PTC)	Christmas factor, antihemophilic factor B, autoprothrombin II, platelet cofactor 2
X	Stuart factor	Prower factor, autoprothrombin III, thrombokinase
XI	Plasma thromboplastin antecedent (PTA)	Antihemophilic factor C
XII	Hageman factor	Glass factor, contact factor
XIII	Fibrin stabilizing factor (FSF)	Laki-Lorand factor (LLF), fibrinase, plasma transglutaminase



Table 10-2. Some Properties of the Coagulation Factors

Factor (1)	Biochemistry (2)	Biosynthesis (3)	Biologic Half-life* (Hours) (4)	Activity in		Function (7)
				Serum (5)	Adsorbed Plasma (6)	
Fibrinogen	Rod shaped glycoprotein, MW 340 000 three globular subunits	Hepatic	72-120	Absent	Unchanged	Precursor of fibrin, common pathway
Prothrombin	Monomeric glycoprotein, MW 69,000, $\alpha$ -2 or $\beta$ Globulin	Hepatic, vitamin K-dependent	72	Absent	Absent	Proenzyme, precursor of thrombin, common pathway
Factor V	MW > 200 000, $\beta$ lipoprotein	Hepatic	?	Absent	Unchanged	Common pathway
Factor VII	$\alpha$ or $\beta$ Globulin, glycoprotein MW ~48-100,000	Hepatic, vitamin K-dependent	12-36 4-6	Absent Increased	Absent	?Proenzyme, extrinsic pathway
Factor VIII	$\alpha$ 2 or $\beta$ Globulin MW > 2,000 000 $\beta$ lipoprotein	?Hepatic TRE system	10-14	Absent	Unchanged	Intrinsic pathway
Factor IX	$\alpha$ or $\beta$ Globulin glycoprotein MW ~50-200 000	Hepatic vitamin K-dependent	24	Increased	Absent	?Proenzyme, intrinsic pathway
Factor X	$\alpha$ Globulin or prealbumin MW ~50-100,000, glycoprotein	Hepatic, vitamin K-dependent	72-60	Unchanged	Absent	Proenzyme, common pathway
Factor XI	$\beta$ or $\gamma$ Globulin, MW ~50-200 000	?Hepatic	746-84	Unchanged	Slightly decreased†	Proenzyme, intrinsic pathway
Factor XII	$\beta$ or $\gamma$ Globulin, MW ~80,000	?	52-60	Unchanged	Unchanged	Proenzyme intrinsic pathway
Factor XIII	$\alpha$ 2-Globulin, MW 320,000, 4 molecular subunits	?Hepatic	72-120	Decreased	Unchanged	Proenzyme; transglutaminase, common pathway

\*Biologic half life, as distinguished from overall in vivo half life or half disappearance time

†Variable, depending on concentration of adsorbent

Question marks indicate insufficient data or significant disagreement between published figures

the study of relatively impure preparations. Most of the information concerning the *in vivo* production, distribution, metabolism, and catabolism of these proteins, i.e., their biodynamics (columns 3 and 4), has been derived from rather inaccurate experiments in patients with hereditary deficiencies of the various factors. Certain biodynamic properties of the coagulation factors are discussed in Chapter 37.

Certain properties of the coagulation factors are included in Table 10-2 primarily because they provide the basis for various *in vitro* tests of coagulation (Chapters 33 and 37), e.g., the presence or absence of the various factors in serum (column 5), their differential absorption by aluminum hydroxide and similar inorganic gels (column 6).

The process of coagulation may be divided into three essentially separate pathways, as described on page 422 (Fig. 10-3). The particular pathway in which the coagulation factors act and the presumed function of each are summarized in Table 10-2, column 7. In the following section, the biochemical and biodynamic properties of the individual factors will be summarized. Details of the interactions between these factors and the properties of their activated forms are discussed in a later section (page 421).

### Fibrinogen

Fibrinogen is a plasma protein that is converted into fibrin in the common pathway of coagulation. Fibrin constitutes the physical basis of all blood clots, and provides the framework for the permanent hemostatic plug (page 398). Fibrinogen also may be essential for normal platelet function (page 392) and wound healing (page 440).

### Biochemistry

Fibrinogen is a relatively insoluble glycoprotein that contains from 3 to 5% carbohydrate.<sup>69,272-414</sup> Purified preparations that are homogeneous by most criteria have been obtained by a number of relatively simple biochemical methods.<sup>70-92,203-268</sup> Such purified fibrinogen is 95 to 97% coagulable by throm-

bin, a figure that approximates the theoretic maximum because 3 to 5% of the molecule is lost as fibrinopeptides during coagulation.<sup>77</sup> The molecular weight of highly purified human fibrinogen is approximately 340,000.<sup>72,92</sup>

Electron microscopy has revealed that the fibrinogen molecule is composed of three nodular subunits, 5 to 7 nm in diameter, interconnected by a thin filament about 1.5 nm thick.<sup>183</sup> Studies of molecular subunits, obtained by dissociating the native protein in reagents such as sodium sulfite<sup>202</sup> or cyanogen bromide<sup>78</sup> suggest that fibrinogen has a dimeric structure (Fig. 10-1).<sup>2,12,74</sup> Each half of the molecule contains three identical pairs of polypeptide chains (designated alpha, beta, and gamma), which have respective molecular weights of 73,000, 60,000, and 50,000.<sup>327</sup> The two halves of the molecule are connected by three *intradimer* disulfide bonds, and the three chains comprising each half are firmly interconnected by *interchain* disulfide bonds, which are concentrated in the N-terminal end of the molecule (*the N-terminal disulfide knots*). These disulfide knots appear to be in the central portion of the molecule,<sup>2</sup> rather than in the lateral nodules as previously supposed.<sup>78</sup> Three pairs of terminal amino acid residues are present.<sup>78</sup> In human fibrinogen, these residues are alanine or asparagiosic (alpha chain), pyroglutamic acid (beta chain), and tyrosine (gamma chain).<sup>73,77</sup>

Two pairs of peptides are removed proteolytically from the fibrinogen molecule by the action of thrombin (Fig. 10-5, II). These have been designated *fibrinopeptides A and B*, and correspond to the terminal ends of the alpha and beta chains (Fig. 10-1). The terminal peptides of the gamma chains are not removed by the action of thrombin. Fibrinopeptide B is chemically homogeneous, but two subtypes of peptide A have been isolated (fibrinopeptides AY and AP).<sup>71,77</sup> Fibrinopeptide B normally is removed by thrombin more slowly than is fibrinopeptide A<sup>74</sup>; the removal of fibrinopeptide B is not required for coagulation.<sup>224</sup>

The venoms of some snakes, including *B. jararaca* (Reptilase) and *A. rhodostoma* (Arvin), contain enzymes that selectively re-

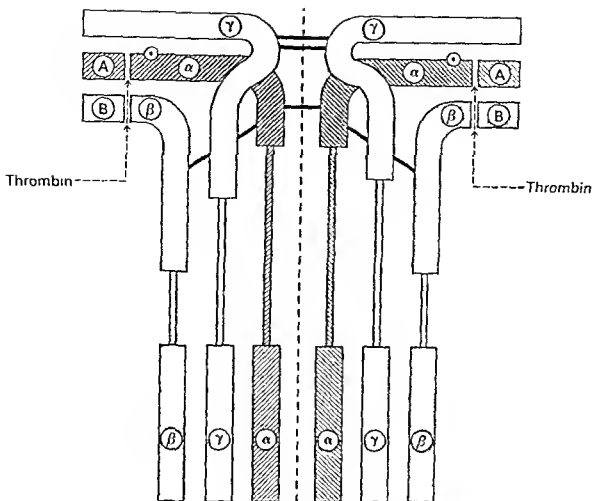


Fig 10-1. The molecular structure of the fibrinogen molecule. The two halves of the fibrinogen molecule (separated by the dotted line) and the constituent three pairs of chains are indicated (diagonal hatched lines = alpha chain, gray = beta chain, white = gamma chain). Stable disulfide bonds between the dimers and between the chains are indicated by solid black lines. Labile disulfides are not illustrated. Also indicated are the two peptides (A, B) cleaved by thrombin (arrows) and the site of the amino acid substitution which is present in fibrinogen *Detroit* (?) (page 1175). Note that the disulfide knots are disproportionately large in the diagram, they comprise only 1.6% of the molecule. (Diagram modified from Wallen<sup>225</sup> and Blombäck<sup>72,73</sup>)

move peptide A.<sup>142,221</sup> The active enzyme in the venom of the southern copperhead (*A. contortrix*) removes fibrinopeptide B more rapidly than fibrinopeptide A.<sup>209</sup>

Peptide sequences of the fibrinopeptides<sup>77</sup> and of larger subunits of the molecule<sup>74,78</sup> have now been determined. Detailed studies of fibrinogens of animal origin have revealed minor but consistent differences from the human protein.<sup>72</sup>

### Biodynamics

Fibrinogen is synthesized by the parenchymal cells of the liver.<sup>156,212,332,492</sup> Approximately 75% of the total body pool of

this abundant protein is present in the plasma,<sup>23,297,500</sup> where the concentration normally ranges from 160 to 415 mg/dl (page 1060). Fibrinogen is present in lesser amounts in lymph,<sup>80,96,194</sup> and may enter the circulation via the hepatic lymphatics.<sup>12</sup> It can be demonstrated in many tissues by immunofluorescent techniques.<sup>166</sup> Infused fibrinogen equilibrates slowly with these extravascular pools.

Extensive studies employing isotopically labeled fibrinogen have revealed that the kinetics of its catabolism are very complicated.<sup>12,34,106,297</sup> The turnover rate of human fibrinogen ranges from 1.7 to 5.0 g/day (30 to 60 mg/kg/day)<sup>25,297,500</sup> As determined in

patients with hereditary afibrinogenemia, the biologic half-life ranges from three to five days.<sup>100,429,544</sup> There is indirect evidence that catabolism occurs continuously<sup>34,500</sup> and may involve the conversion of fibrinogen into soluble derivatives of lower molecular weight; these have been isolated from normal plasma (fraction I-8).<sup>188,342,463</sup> The *in vivo* sites of fibrinogen catabolism are unknown.

The maximal production rate of fibrinogen may increase greatly under experimental conditions.<sup>261</sup> There is indirect evidence that plasma levels of fibrinogen degradation products, possibly acting as a "feed back" control, may constitute the major regulators of the rate of fibrinogen synthesis.<sup>261</sup> In tissue culture, free fatty acids increase fibrinogen synthesis by human liver slices.<sup>353</sup>

### The Vitamin K-Dependent Coagulation Factors

#### Vitamin K

Studies by several groups culminated almost simultaneously in the 1930's in the identification of vitamin K, the recognition of its importance as an "anti-bleeding factor" in obstructive jaundice, and the development of a specific pharmacologic antagonist of its action. To a large extent, subsequent studies have only amplified these remarkable pioneering endeavors.<sup>99,514</sup>

#### Pharmacology

In nature, vitamin K exists in two forms (Fig. 38-1, page 1203), i.e., vitamin K<sub>1</sub> (phytylquinone), which is found in various vegetable oils and leafy plants, and the vitamins K<sub>2</sub> (menaquinones), a group of closely related compounds that are synthesized by various bacteria, including common gut flora. It has been difficult to establish dietary requirements for this vitamin.<sup>158</sup> There is evidence in both animals and man that the vitamin K formed in the colon, where most is synthesized, is not absorbed, and the importance of dietary sources of this vitamin may be greater than previously realized.<sup>512</sup>

Both vitamin K<sub>1</sub> and the vitamins K<sub>2</sub> are fat soluble, and as a consequence are absorbed only in the presence of bile salts. They are carried in the plasma bound to albumin. No significant body stores of either form of vitamin K have been demonstrated.<sup>99</sup>

The coumarin-like drugs inhibit the physiologic action of vitamin K, and are discussed in detail on page 1243.

#### Function

Vitamin K is required for the biosynthesis of four coagulation factors: prothrombin and factors VII, IX, and X. Reports that factor XI also is vitamin K-dependent have not been confirmed.<sup>415</sup> The essential role of vitamin K in the synthesis by the hepatic cell of these coagulation factors has been demonstrated directly in liver cell homogenates, slices, and isolated microsomes,<sup>42,245</sup> as well as by means of extracorporeal perfusion of the intact liver.<sup>121,380,405,410</sup> In the case of factor IX, the results of liver perfusion have been equivocal.<sup>380,405</sup>

The biochemical mode of action of the vitamins K remains obscure. These vitamins are not incorporated into any circulating coagulation factor, and data pertaining to the effects of vitamin K on mitochondrial electron transport and oxidative phosphorylation in bacteria are of uncertain significance in man.<sup>99</sup> Evidence obtained from the study of the inhibitory effects of coumarins, and non-specific inhibitors of protein synthesis such as actinomycin and puromycin,<sup>36,211</sup> suggests that the vitamins K catalyze the last of at least two synthetic steps leading to the production of the various coagulation factors (Fig. 10-2). Consistent with this view is evidence that coumarin drugs and vitamin K deficiency induce the formation of abnormal molecular analogs of the vitamin K-dependent coagulation factors. These may represent a common precursor, or "incomplete" individual coagulation factors. Detailed biochemical studies of "dicoumarol-induced" prothrombin of cows<sup>348,487,488</sup> have demonstrated that this totally nonfunctional protein is identical to normal prothrombin with respect to its elec-

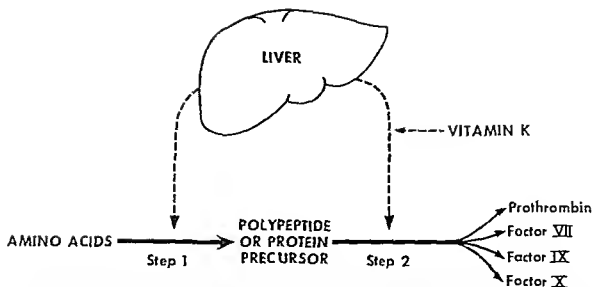


Fig. 10-2. Two-step hypothesis for biosynthesis of vitamin K-dependent coagulation factors. A solid arrow denotes transformation, a dashed arrow denotes action. When step two is inhibited as a result of deficiency or inhibition of vitamin K, the precursors produced in step 1 accumulate. This would be marked if the "feedback" that regulates the synthetic rate acts primarily on step 1.<sup>35,196</sup>

trophoretic mobility, amino acid composition, and major antigenic determinants, but lacks  $\text{Ca}^{++}$  binding sites. These data are consistent with the hypothesis that the second, vitamin K-dependent biosynthetic step (Fig. 10-2) involves the formation of  $\text{Ca}^{++}$  binding sites on the prothrombin molecule. Contrary to earlier hypotheses,<sup>35,196</sup> this step does not appear to involve the attachment of a sugar or glycoprotein moiety.<sup>316,487,488</sup> Other substances that may be related to "dicoumarol-induced" prothrombin include the "protein induced by vitamin K absence or antagonism (PIVKA)," a functionally inert protein that is a competitive inhibitor of factor X,<sup>196</sup> and "pre-prothrombin," a substance that resembles prothrombin biochemically, but is activated *in vitro* only by staphylocoagulase.<sup>199,248,478</sup> Qualitatively abnormal forms of factors VII, IX, and X also have been demonstrated by immunologic methods.<sup>111</sup>

### Prothrombin

Prothrombin is a proenzyme, the precursor of thrombin, that functions in the common pathway of coagulation.

Highly purified prothrombin has been prepared from both human and bovine plasma.<sup>27,236,308,309,504</sup> Human prothrombin has a molecular weight of 69,000 and contains from 2 to 10% carbohydrate.<sup>248,274</sup> It migrates electrophoretically as an alpha-2 or beta globulin,<sup>110,247</sup> and contains one disulfide bridge<sup>308</sup> and a single terminal amino acid residue (alanine).<sup>274,308</sup> It has not been successfully dissociated into constituent subunits, and apparently is composed of a single polypeptide chain. The amino acid content of prothrombin has been determined.<sup>303,274</sup>

Prothrombin is present in human plasma in concentrations of approximately 10 to 15 mg/dl.<sup>140</sup> A major portion of the total body pool of this proenzyme is in the lymph<sup>89,96,491</sup> and other intravascular sites.<sup>461</sup> When purified <sup>131</sup>I-labeled prothrombin was infused into normal subjects, its disappearance was biphasic, with a rapid component ( $t_{1/2} = 8$  hours) reflecting equilibration with extravascular pools, followed by a slower component ( $t_{1/2} = 2.8$  days) that represents the true biologic half-life. The turnover rate was 2.4 mg/kg/day.<sup>461</sup> Similar figures have been obtained in patients with hereditary hypoprothrombinemia<sup>461</sup> (page 1177).

The hypothesis proposed by Seegers and his collaborators concerning the nature and function of prothrombin is discussed on page 426.

### Factor VII

Factor VII functions together with tissue factor in the extrinsic pathway of coagulation. The nature of the active form of this protein is uncertain. Studies of factor VII in preparations concentrated up to 8,000-fold have revealed a molecular weight that ranges from 48,000 to 100,000, depending on the source material and the exact methods employed.<sup>40,409</sup> Factor VII contains an unusually high concentration of carbohydrate, as much as 50% in some preparations<sup>409</sup>; it migrates electrophoretically as an alpha or beta globulin.<sup>383</sup> Its concentrations in human plasma have been estimated at 3 mg/dl<sup>410</sup>; it is also detectable in lymph.<sup>80,96,494</sup> There is preliminary evidence that this factor, or a subunit or precursor thereof, is produced or stored in the kidney.<sup>121,410</sup>

Factor VII apparently has the most rapid biologic turnover rate of any plasma protein. In patients with hereditary factor VII deficiency, the disappearance of infused factor VII is biphasic, and the biologic half-life ranges from four to six hours.<sup>82,215,316</sup> The reasons for the rapid catabolism are obscure.

### Factor IX

Factor IX is involved in the intrinsic pathway of coagulation. Evidence regarding the nature of its active form is inconclusive. Despite intensive effort, this protein has not been extensively purified or characterized chemically.<sup>392</sup> Purified bovine factor IX has a molecular weight of 55,000, and is composed of a single polypeptide chain.<sup>158a</sup> It contains approximately 26% carbohydrate.

Approximately 60% of the total body pool of factor IX is apparently extravascular.<sup>216</sup> This factor has been demonstrated in lymph,<sup>80,96,491</sup> but is present in the plasma in only trace amounts. Biphasic survival

curves with rapid and slow components are obtained when factor IX is infused into patients with hereditary deficiencies of this substance. The biologic half-life, as determined in such studies, is approximately 24 hours.<sup>61,216,330</sup> Estimates of the turnover rate obtained with <sup>35</sup>S-labeled plasma suggested much slower catabolism.<sup>11</sup>

### Factor X

Factor X is a proenzyme that is essential for the formation of prothrombinase in the common pathway of coagulation. It is activated by the products of both the intrinsic and extrinsic pathways.

The electrophoretic mobility of factor X ranges from that of an alpha globulin to that of a prealbumin.<sup>136</sup> Estimates of the molecular weight of human factor X have ranged from 50 to 100,000, depending on the purification method employed.<sup>283</sup> This variation may be the result of the tendency of this protein to aggregate in the presence of divalent cations.<sup>136</sup> Bovine factor X has a molecular weight of 55,000, and contains 10% carbohydrate.<sup>159,239</sup> It is composed of two polypeptide chains that have molecular weights of 38,000 and 17,000, respectively. All of the carbohydrate and the active serine moiety are found on the heavy chain; the light chain appears to be essential for the binding of factor X to phospholipids. Other studies<sup>312</sup> suggest that the native molecule is a single polypeptide chain. Bovine factor X can be chromatographically separated into two chemically different but functionally similar forms, which have been designated factors X<sub>1</sub> and X<sub>2</sub>.<sup>149,239</sup>

The biologic half-life of factor X, as determined in patients with hereditary deficiencies of this factor, ranges from 24 to 60 hours.<sup>61,82,379,437</sup> An initial rapid component was evident in the survival curves ( $t_{1/2} = 2-9$  hours). This observation, together with the presence of factor X in lymph,<sup>80,96,491</sup> implies the existence of significant extravascular pools. The plasma concentration of factor X has been estimated at 1.2 mg/dl.<sup>410</sup>

### Factor V

Factor V is essential for the formation of prothrombin in the common pathway of coagulation. The biochemical role of this trace protein in coagulation remains unclear.

The purification of factor V has proved difficult because of its *in vitro* lability. The molecular weight of purified human factor V is in excess of 200,000<sup>283</sup>; the bovine protein has a molecular weight of 290,000.<sup>139</sup> Active molecular subunits have been isolated by gel filtration; the larger fragments apparently contain phospholipids.<sup>47,397</sup> Factor V migrates electrophoretically as an albumin.

The *in vitro* activity of factor V is increased by the action of thrombin (page 429), Russell's viper venom,<sup>445</sup> and papain.<sup>401</sup> Factor V is unstable when stored in citrated plasma, and is rapidly inactivated *in vitro* by strong chelating agents such as EDTA and oxalate (page 430).

Factor V is synthesized in the liver.<sup>350</sup> In patients with hereditary deficiencies of this factor, the initial *in vivo* recovery of infused factor V is virtually complete, and the survival curve is monophasic.<sup>82,529</sup> Although these observations suggest the absence of significant extravascular depots of factor V, this factor has been identified in lymph.<sup>60,86,494</sup> Estimates of its biologic half-life have varied greatly, ranging from 12 to 36 hours.<sup>82,529</sup>

### Factor VIII

Factor VIII is a trace protein that is involved in the intrinsic pathway of coagulation. Its precise biochemical function in coagulation remains uncertain.

#### Biochemistry

Efforts to purify factor VIII have been hampered by its *in vitro* lability,<sup>79</sup> and difficulties in separating this protein from fibrinogen. It may be concentrated by cryoprecipitation (page 1186) and by gel filtration.<sup>425</sup> Studies of material purified up to 10,000-fold

with respect to plasma<sup>55,56,206,229,312,524</sup> suggest that the molecular weight of factor VIII is on the order of 2,000,000 or even higher, and that the molecule contains both carbohydrate<sup>207,254</sup> and lipid moieties.<sup>76,208</sup> Some investigators consider the lipid to be a contaminant.<sup>428</sup> The electrophoretic mobility of factor VIII is that of an alpha-2 or beta globulin.<sup>206</sup> Its activity is increased by certain phospholipases<sup>208</sup> and by low concentrations of thrombin (page 429). Like factor V, factor VIII is unstable in citrated plasma, and is rapidly inactivated by strong chelating agents.<sup>533</sup> (page 430).

Several workers have demonstrated the presence of active molecular subunits of factor VIII.<sup>206,534,535</sup> Those derived from the human protein have a molecular weight of approximately 250,000.<sup>312</sup> Bovine factor VIII may be dissociated into smaller subunits of molecular weight 103,000<sup>206</sup> which may be coupled to a larger "carrier" molecule in the circulation.<sup>355</sup> Preliminary studies have suggested that the major antigenic determinants of factor VIII may be located on a different molecular subunit than the active site concerned with coagulant activity. The precursor or subunit containing the antigenic sites is identical or related to the "anti-bleeding factor" which is deficient in von Willebrand's disease (page 1179).

#### Production

Despite extensive study, the sites of production of factor VIII have yet to be defined.<sup>206</sup> Most investigations have been concerned with the roles of the liver, the spleen, and the reticuloendothelial system.

Transplantation experiments in hemophilic dogs<sup>313,326,361,531</sup> provide evidence that factor VIII is synthesized in the liver. Thus, the transplantation of a normal liver into a hemophilic dog produced a significant increase in the factor VIII levels of the recipient animal<sup>313,531</sup>; in one experiment, levels in excess of 50% of normal persisted for as long as 140 days.<sup>313</sup> Hepatic venous blood contains more factor VIII than peripheral venous

blood.<sup>164</sup> There is little additional evidence for hepatic synthesis of factor VIII. Studies utilizing techniques such as specific immunofluorescence, experimental hepatic injury and extirpation, and extracorporeal<sup>121</sup> or in situ organ perfusion<sup>164</sup> have yielded inconclusive or contradictory results. The presence of normal or even high levels of factor VIII in patients with severe hepatocellular disease suggests that the hepatic cells are not the source of this factor. It may, however, be produced by the reticuloendothelial cells in the liver.

The importance of the *spleen* in the bio-dynamics of factor VIII is suggested by several observations. Thus, a significant increase in the plasma level of factor VIII has been demonstrated in both humans and dogs following epinephrine administration,<sup>234,434</sup> a response that is lacking in asplenic subjects.<sup>286</sup> Both heterotopic and orthotopic spleen transplants produce significant but transitory increases in the factor VIII levels of hemophilic dogs.<sup>313,326,361,531</sup> The results of splenic transplantation in one hemophilic patient were similar.<sup>192</sup> Splenectomy, however, does not affect the factor VIII levels of normal or hemophilic dogs or humans.<sup>178</sup> This evidence is compatible with the hypothesis that splenic synthesis of factor VIII is of minor physiologic importance, although the spleen may act as a significant storage site for this coagulation factor. Results of experiments in which asplenic normal dogs were cross-circulated with hemophilic dogs are consistent with this view.<sup>528,531</sup> Studies of organ perfusates suggest that the liver may produce a substance that "induces" factor VIII synthesis by the spleen.<sup>122</sup>

In one study, the infusion of homogenates of normal spleen into patients with factor VIII deficiency had the surprising effect of significantly increasing the factor VIII levels of the recipients for two to three months.<sup>113</sup> In another, in vitro factor VIII synthesis was attributed to normal lymphocytes and fibroblasts.<sup>548</sup> Both of these studies require confirmation.

The *reticuloendothelial system* as a whole has been implicated as the site of factor VIII

synthesis.<sup>530</sup> This hypothesis is difficult to prove or refute. The effects of total body irradiation on factor VIII levels of animals have been inconsistent,<sup>395,473,530</sup> and are difficult to interpret in any case. The transplantation of normal marrow into hemophilic dogs does not increase factor VIII levels,<sup>531</sup> even if the marrow graft is completely successful.<sup>490</sup>

Preliminary studies have suggested that the *kidney* also may participate in some way in the synthesis of factor VIII.<sup>45,121</sup> Thus, a small molecular weight protein, which exhibits factor VIII activity in vitro, has been isolated from kidney homogenates.<sup>45</sup> It was postulated that this protein may be a precursor or a subunit of factor VIII. Various succinylated proteins, including albumin,<sup>46</sup> have factor VIII-like activity in vitro. It has been suggested that "kidney factor VIII" represents such a protein.<sup>200</sup>

Information obtained from the study of von Willebrand's disease suggests that a humoral factor is in some way involved in factor VIII biosynthesis. This is discussed on page 1181.

In summary, available data would suggest that factor VIII originates in a system of cells that may be found in various organs. The liver appears to be the major source of this protein; the spleen apparently is the major storage site for this factor. It is not improbable that factor VIII production involves an interaction between two or more cellular or organ systems.

### Distribution and Turnover

The plasma concentration of factor VIII is on the order of 1  $\mu\text{g}/\text{dl}$ .<sup>140</sup> The in vivo distribution of this protein is poorly understood. The initial disappearance rate of factor VIII infused into hemophilic patients is rapid ( $t_{1/2} = 4$  to 6 hours). It has been assumed that this is due to the equilibration of the infused factor VIII with extravascular pools,<sup>11,470</sup> but there is little evidence for this hypothesis. Only small amounts of factor VIII are present in lymph,<sup>80,96,494</sup> and, in view of the large size of the molecule, rapid



extravascular distribution of infused factor VIII would seem unlikely.<sup>206</sup> The adsorption of factor VIII by platelets, other cells, or endothelium may explain this phenomenon, at least in part.

The slow component of the survival curve has a half-life of approximately 12 hours, and ranges from 8 to 18 hours in individual patients.<sup>3,82,406,470</sup> In many hemophiliacs, triphasic survival curves have been described, eg, a biphasic rapid initial loss of factor VIII, a rise following an initial rapid decrement of activity.<sup>3,390</sup> Data obtained with an *in vivo* protein labeling technique that employs <sup>35</sup>S-methionine suggest a much slower turnover rate for factor VIII (biologic  $t_{1/2}$  = 2.9 days) than do studies carried out in hemophilic patients. This discrepancy may be explained by the persistence within the circulation of inactive factor VIII.<sup>41</sup> Similar differences have been noted between the *in vivo* survival time of immunologically active factor VIII, and of functionally active factor VIII (page 1181).

### Factor XI

Factor XI is a proenzyme that is essential in the intrinsic pathway of coagulation. Surprisingly little is known concerning its biochemistry.<sup>187,369</sup> It is a trace protein that migrates electrophoretically as a beta or gamma globulin, and its molecular weight has been estimated at 50,000 to 200,000.<sup>140,285</sup> Its activated form may be eluted from the surfaces of particulate silicates such as celite.<sup>365</sup> Soybean trypsin inhibitor blocks the *in vitro* esterolytic activity of factor XIa, but does not affect its coagulant activity,<sup>359</sup> an observation which suggests that the proteolytic activity of factor XIa may not be related to its role in coagulation.

Plasma levels of factor XI frequently are diminished in patients with liver disease<sup>415</sup> (page 1205), but direct evidence for hepatic biosynthesis of this protein is lacking. Estimates of its biologic half-life obtained in patients with hereditary deficiency states have ranged from 48 to 84 hours.<sup>371,372,439</sup>

### Factor XII

Factor XII is activated by contact with foreign surfaces, and initiates the intrinsic pathway of coagulation. It also is involved in the activation of fibrinolysis (page 432) and in the plasma kinin system (page 440).

Factor XII of human origin has been purified extensively.<sup>423</sup> Its molecular weight is approximately 80,000,<sup>179</sup> and it migrates electrophoretically as a beta or gamma globulin.<sup>418</sup> Factor XII of bovine origin is a sialoglycoprotein,<sup>446</sup> but no carbohydrate was found in purified preparations of human factor XII.<sup>420</sup> Available evidence suggests that the *in vitro* esterolytic activity of this protein, like that of factor XIa, may be unrelated to its coagulant activity.<sup>419,423,468</sup>

The *in vivo* sites of synthesis of factor XII are unknown. The plasma levels of this factor usually are normal even in severe liver disease. The biologic half-life, as estimated in patients with hereditary deficiencies, ranges from 52 to 60 hours.<sup>248,515</sup>

### Factor XIII

The enzymatic form of factor XIII acts in the common pathway of coagulation where it forms stabilizing covalent bonds within fibrin strands. Factor XIII also may be involved in wound healing (page 440).

Factor XIII purified from human plasma is an alpha-2 globulin with a molecular weight of approximately 320,000. This protein can readily be dissociated into two pairs of subunits, ie, two alpha chains (MW 75,000) and two beta chains (MW 88,000).<sup>289,291,293,447</sup> It also is present in the platelets, and apparently is synthesized by the megakaryocytes<sup>250</sup> (page 378). Platelet factor XIII is composed of only two alpha chains, and has a molecular weight of 146,000.<sup>324,447</sup> Substances that exhibit factor XIII activity *in vitro* are present in a number of other tissues, and a transglutaminase, which is apparently unrelated to factor XIII of plasma origin, is found in liver homogenates.<sup>144,509</sup>

Plasma factor XIII is deficient in many patients with liver disease (page 1205), but

direct evidence for its hepatic biosynthesis is lacking. It has been suggested that this factor may be activated by the liver, or that an inhibitor of factor XIII arises as the result of liver disease.<sup>374</sup>

The biologic half-life of plasma factor XIII, as determined in patients with hereditary deficiency states, ranges from three to five days.<sup>87,233</sup>

### Tissue Factor

Homogenates of normal tissues markedly accelerate blood coagulation, a fact that was well known in the last century.<sup>304</sup> The responsible substance is termed "tissue factor" (factor III, thromboplastin).<sup>94</sup> It functions in coagulation by interacting with factor VII in the extrinsic pathway (Fig. 10-4).

The biochemistry of tissue factor is poorly understood.<sup>94,352</sup> It is a particulate complex of phosphatides, lipoproteins, and cholesterol,<sup>341,401</sup> which has a molecular weight of approximately 425,000.<sup>350</sup> Active tissue factor may be prepared from virtually any tissue; in homogenates it is found in the microsomal fraction.<sup>53,98</sup>

Tissue factor may be dissociated into its constituent lipoproteins and phosphatides by solvent extraction. Neither fraction is active in coagulation alone, but, when the two are recombined, full activity is restored.<sup>98,193,350</sup> The phosphatide fraction is composed of variable amounts of sphingosine, phosphatidylinositol, phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine.<sup>349</sup> The relative concentrations of these phosphatides do not appear to be critical insofar as the coagulant action of the complex is concerned; the structure and charge of the lipid moiety may be more important.<sup>168</sup> The action of tissue factor in coagulation is species specific, a characteristic that resides entirely in the protein moiety.<sup>237,350</sup> The relative coagulant activity of heterologous tissue factors in human plasma may have diagnostic significance, eg, in some of the variants of factor IX deficiency (page 1172). Some preparations of tissue factor exhibit enzymatic activity *in vitro*,<sup>352,402,412</sup> but this may be unrelated to their coagulant action.<sup>251</sup>

### Miscellaneous Coagulation Factors

There is evidence for the existence of several substances, in addition to the coagulation factors already discussed, that are active in coagulation. These include the Fletcher factor,<sup>190</sup> the Carr factor,<sup>95</sup> the Nishimine factor,<sup>546</sup> the Tatsumi factor,<sup>347</sup> the Dynia abnormality,<sup>302</sup> "thorium-vulnerable" factor,<sup>103,214</sup> "prephase" accelerator,<sup>149</sup> thromboplastin generation accelerator,<sup>389</sup> the "ADP-sensitive" factor,<sup>403</sup> and others.<sup>104,175</sup> Inherited disorders due to deficiencies of these "factors" are discussed on page 1183.

### Variations of Coagulation Factors in Health and Disease

#### Neonates

A moderate deficiency of the vitamin K-dependent coagulation factors (prothrombin and factors VII, IX and X) is present at birth.<sup>1,190,381</sup> Observed plasma levels vary widely, but usually range from 20 to 50% of those normally found in adults.<sup>1,180</sup> This apparently is the result of immaturity of the biosynthetic apparatus in the liver, and is unaffected by vitamin K given to the mother. The levels of these four factors fall even further during the first two to four days of the infant's life. This "secondary fall" is prevented if vitamin K is administered to either the mother or infant, and appears to represent the effects of a transient "physiologic" deficiency of the vitamin, which is probably attributable to a sterile gut or the absence of oral vitamin intake. Exaggeration of this phenomenon underlies hemorrhagic disease of the newborn (page 1201). Deficiencies of the vitamin K-dependent factors often are more pronounced in premature than in full-term infants, and vary in inverse proportion to gestational age and birth weight.<sup>88</sup>

Other abnormalities of coagulation may be "physiologic" in the newborn.<sup>1</sup> Thus, a qualitatively abnormal form of fibrinogen has been isolated from the plasma of normal newborns. Such *fetal fibrinogen* clots at an abnormally slow rate and differs chemically from adult fibrinogen in several respects.<sup>541</sup>

It disappears from the circulation during the first months of life.

Deficiencies of *factor XII*<sup>264</sup> and *factor XI*,<sup>210,372</sup> with levels ranging from 25 to 50% of adult norms, have been reported in normal infants; these deficiencies disappear within two to four weeks.<sup>264</sup> Assays for *factor XIII* have yielded variable results in the newborn.<sup>150</sup>

None of the coagulation abnormalities described above is of clinical significance in the normal neonate.

Plasma levels of *fibrinogen*, *factor V*, and *factor VIII* approximate adult norms in the normal neonate or thriving premature infant.<sup>1,189,373,381,457a</sup>

### Miscellaneous Variations

Virtually any disorder associated with stress, inflammation, or tissue necrosis may produce *hyperfibrinogenemia*. This may develop within a matter of hours, and is the major factor leading to an acceleration of the erythrocyte sedimentation rate in such disorders (page 125). *Hyperfibrinogenemia* also is seen in pregnancy,<sup>253</sup> following the administration of anovulatory medications,<sup>85</sup> and in hypermetabolic states.<sup>217</sup>

Increased plasma levels of *factor VIII* have been well documented in a large variety of situations.<sup>217,253</sup> Significant increases are consistently associated with various hypermetabolic states,<sup>217,472</sup> and have been attributed to hypersensitivity to catecholamines of endogenous origin.<sup>472</sup> *Factor VIII* levels also rise following vigorous exercise<sup>433</sup> and epinephrine administration.<sup>231,433</sup> The latter response presumably is the consequence of the release of *factor VIII* from the spleen.<sup>286</sup> A progressive rise in *factor VIII* levels is seen in pregnancy,<sup>253</sup> and persistent elevations are common in patients with chronic thrombocytopenia<sup>286</sup> and in those receiving oral contraceptives.<sup>108</sup> Neither splenectomy nor corticosteroids affect *factor VIII* levels.<sup>90</sup> Numerous other disorders have been associated with moderate increases in *factor VIII* levels.<sup>4,286</sup> The plasma levels of this factor normally are slightly greater in men than in women, and in persons of blood group A.<sup>407</sup>

The levels of the *vitamin K-dependent factors* are elevated in disorders associated with hypermetabolism, with the exception of *factor IX*, which is normal in hypermetabolic states even though it is diminished in myxedema.<sup>287,472</sup> These changes are associated with corresponding alterations in the turnover rate of these factors.<sup>287</sup> The levels of *factors VII, IX, and X* are increased in pregnancy, but prothrombin is little affected.<sup>253</sup> The administration of estrogenic and progestational hormones increases the levels of these factors to a variable extent.<sup>85,471</sup>

*Factor V* levels are unaffected by pregnancy<sup>253</sup> or hyper- or hypometabolism,<sup>472</sup> and are slightly if at all increased following exercise or epinephrine administration.<sup>157,232</sup>

*Factor XI* levels are diminished in hypothyroidism, but are normal in hyperthyroidism.<sup>472</sup> Plasma levels of this factor fall gradually during pregnancy.<sup>373</sup> Levels of *factors XI* and *XII* are markedly increased by exercise.<sup>132,231</sup> *Factor XIII* may decrease during normal pregnancy.<sup>108</sup>

## The Physiology of Blood Coagulation

The theories that have been proposed to explain the phenomena of blood coagulation are so numerous and varied that even a brief discussion of each is beyond the scope of the present section.<sup>59,62,110,137,304</sup> The discussion to follow will be based on a hypothesis that was independently presented in 1964 by MacFarlane<sup>200</sup> and by Davie and Ratnoff,<sup>109</sup> i.e., the "cascade" or "waterfall" hypothesis.

As originally conceived, this hypothesis viewed coagulation as an interlinked sequence of proenzyme-to-enzyme transformations.<sup>300</sup> Coagulation factors, which normally exist in the plasma as inert precursors, are transformed into enzymes when activated. These enzymes then convert the precursor next in line into its enzymatic form (Fig. 10-4, reactions 1, 2, 3). Each coagulation factor thus acts first as a substrate and then as an enzyme. The ability of small amounts of enzyme to activate large amounts of substrate in each successive step in the process was viewed as

a "biologic amplifier" of possible homeostatic importance.<sup>140</sup>

From the outset, the limitations of this formulation were apparent to its originators,<sup>301</sup> and several revisions of the coagulation "cascade" have since been made because of new information.<sup>301</sup> Despite continuing disagreement concerning the details of individual steps, the essential core of the cascade hypothesis remains widely accepted. Although highly theoretical, the scheme summarized in Figure 10-4 explains most available data, and continues to serve as a useful framework upon which new observations may be oriented.

### Pathways of Coagulation

Coagulation is initiated by two fundamentally different mechanisms, ie, the process of contact activation and the action of

tissue factor. It initially proceeds by two separate pathways that "converge" by activating a third common pathway leading to fibrin formation (Fig. 10-3).

Contact activation initiates a series of reactions involving factors XII, XI, IX, and VIII and platelet factor 3 (PF-3). These reactions lead to the formation of an enzyme that activates factor X; they proceed normally in the absence of tissue factor, and do not involve factor VII.<sup>10</sup> They are designated the *intrinsic pathway*.

When coagulation is initiated by tissue factor, an interaction between this substance and factor VII leads to the production of an enzyme that also activates factor X. This relatively simple process proceeds normally in the absence of factors XII, XI, VIII, and IX and PF-3, and does not require contact activation. It is termed the *extrinsic pathway*.

Subsequent steps in the process of coagu-

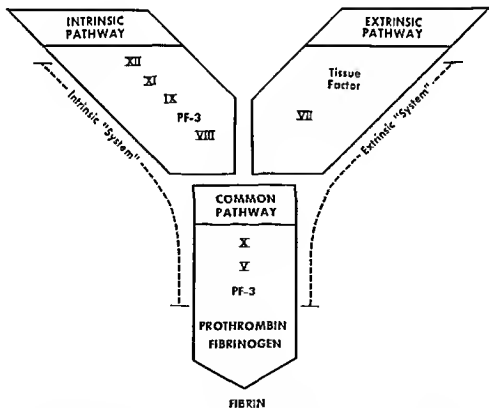


Fig 10-3. Pathways of coagulation. The terms *intrinsic* and *extrinsic "system"* are widely used with reference to the reactions indicated by dashed lines. PF-3 denotes platelet factor 3.

lation involve factors X and V, PF-3, prothrombin and fibrinogen. They proceed in essentially the same way whether factor X is activated by the product of the intrinsic pathway or the product of the extrinsic pathway. These reactions are termed the *common pathway*.

## The Intrinsic Pathway

### The Contact Phase

The phenomenon of *contact activation*<sup>219,522</sup> (*reaction 1*, Fig. 10-4) involves the adsorption of factor XII upon any of a variety of "active" surfaces, and results in a change in the conformation of this protein.<sup>125,365</sup> The nature of this change is uncertain, but it may involve an uncoiling of the molecule leading to the exposure of an enzymatically active site.<sup>184,521</sup>

Contact activation does not require calcium ions, and is produced in vitro by a variety of electronegative surfaces,<sup>228</sup> eg, glass, asbestos, particulate silicates such as Celite or kaolin, spider webs.<sup>369</sup> The amount of factor XII activated is proportional to the surface area of the activator. Thus, much greater activation results from a particulate activator than from a glass tube. Biologic surfaces that produce contact activation include collagen fibers,<sup>540</sup> unbroken skin,<sup>367</sup> sebum, long chain fatty acids,<sup>119,320</sup> uric acid,<sup>256</sup> homocystine,<sup>419</sup> and possibly fibrin and elastin.<sup>360</sup> Collagen presumably plays a major role in vivo. Chemical alterations of collagen that block or remove the free carboxyl groups of glutamic and aspartic acid abolish the ability of collagen to induce contact activation.<sup>540</sup> There is good experimental evidence that optimally spaced foci of negative charges are the critical determinants of both natural and artificial "active" surfaces.<sup>369</sup>

The nature of the interaction between factors XIIa and XI is unclear and controversial. This reaction occurs in the absence of ionic calcium, and involves the conversion of factor XI into XIa by factor XIIa. There is much to suggest that factor XIIa acts as an enzyme

in this reaction<sup>421</sup> (*reaction 2*), although the evidence is not conclusive.<sup>369,446</sup> Supporting evidence for this view has been obtained with purified factors XII and XI.<sup>421,427</sup> It has also been proposed that factors XIIa and XI interact to form a complex on the active surface, which then activates subsequent steps.<sup>179,422</sup> A third alternative is that factor XI functions as a cofactor for the enzymatic action of factor XIIa.<sup>137</sup>

There is general agreement that the product of the interaction between factors XIIa and XI (often termed *activation* or *contact product*) acts as an enzyme,<sup>301,369</sup> whatever its exact nature may be. In the next step (*reaction 3*), this substance and factor IX interact. Studies of the kinetics and stoichiometry of this reaction suggest that factor IX behaves as a substrate.<sup>93,368,442,443</sup> The reaction requires calcium ions, and is inhibited by heparin.

### The Activation of Factor X

The next step in the intrinsic pathway is the interaction between factor IXa, factor VIII, and PF-3 (*reaction 4*, Fig. 10-4). This step has proved very difficult to study, and remains poorly understood. There is general agreement on the following points: (1) it is calcium-dependent; (2) it requires traces of PF-3 or a platelet substitute<sup>444</sup>; (3) it is markedly accelerated by the action of traces of thrombin on factor VIII (page 429); and (4) it leads to the evolution of an enzymatic activity capable of activating factor X.

In the original cascade hypothesis<sup>300</sup> it was proposed that factor IXa converted factor VIII into its active form, which then activated factor X. Although this hypothesis cannot be dismissed, evidence for the existence of factor VIIIa or for the enzymatic activity of factor IXa is not entirely convincing.<sup>296,307,382</sup> Most evidence favors the view that factor IXa, factor VIII, calcium ions, and PF-3 form a complex by means of a unique physicochemical interaction.<sup>194,226</sup> In terms of this hypothesis, the phospholipid serves as a surface upon which coagulation factors and ions are adsorbed and oriented in such a

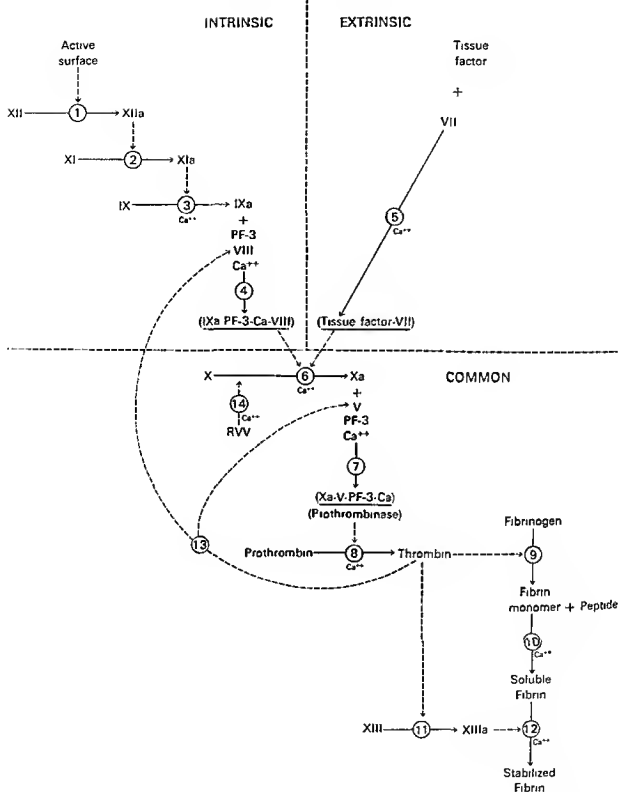


Fig. 10-4. The interactions of the coagulation factors. A modification of the cascade or waterfall hypothesis of MacFarlane<sup>100</sup> and of Davie and Ratnoff.<sup>101</sup> The three pathways of coagulation (Fig. 10-3) are separated by dashed lines. A solid arrow indicates transformation; a dashed arrow denotes action. Complexes are underlined and enclosed in parentheses, eg **(Xa V PF-3-Ca)**. Reaction numbers, indicated within arrows, are referred to in the text. The "autocatalytic" actions of thrombin (reaction 13) are illustrated in dotted lines. RVV is an abbreviation for Russell's viper venom (reaction 14).

manner that the resulting complex acquires enzymatic activity.<sup>97,200,382</sup> This reaction is quite similar to that believed to involve PF-3, factor V, and factor Xa in the common pathway (reaction 7).

Alternative hypotheses are quite consistent with most of the data. For example, factor IXa may directly activate factor X,<sup>137</sup> factor VIII acting as a cofactor.

### The Extrinsic Pathway

The extrinsic pathway involves only the interaction between tissue factor (factor III) and factor VII (reaction 5, Fig. 10-4). This requires the presence of calcium ions, and leads to the formation of a complex that is particulate<sup>91</sup> and behaves as an enzyme.<sup>348,369,539</sup> Studies of semi-purified preparations of bovine factor VII and tissue factor suggest that each of these components has discrete enzymatic activity,<sup>352,402</sup> but that the coagulant activity of the complex formed between them is the result of an autocatalytic degradation of factor VII that occurs following the attachment of factor VII to tissue factor.<sup>351</sup> An alternative view is that the major enzymatic activity resides in an "activated" form of tissue factor, and that factor VII acts mainly as a cofactor.<sup>137</sup>

There is some evidence that the extrinsic and intrinsic pathways interact. Thus, activation product, formed during the contact phase of the intrinsic pathway, appears to activate the extrinsic pathway by means of a poorly understood interaction with factor VII.<sup>22,244,459</sup> The contact factors also may interact with platelets, leading to the activation of the extrinsic pathway, as discussed on page 397. Activation of the intrinsic pathway by tissue factor and factor VII also may occur (page 439).

Since tissue factor contains phospholipids that act as platelet substitutes, additional PF-3 from platelets is not required for fibrin formation when coagulation is initiated by the extrinsic pathway, eg, in the measurement of the plasma prothrombin time (page 1056).

## The Common Pathway

### Formation of Prothrombinase

The common pathway of coagulation begins with the activation of factor X (reaction 6, Fig. 10-4). This step is calcium-dependent, and involves a proenzyme-to-enzyme transformation which is accomplished by the two different enzymatic activities that evolve from the reactions of the intrinsic and extrinsic pathways, respectively, as discussed above. The activation of factor X involves the cleavage of a peptide bond and the release of a glycopeptide of molecular weight 11,000.<sup>222</sup> Factor X also is activated by Russell's viper venom in the absence of other coagulation factors (reaction 14).<sup>141,299</sup> The active principle, purified from the crude venom, is a protease of small molecular weight.<sup>445,538</sup>

Activated factor X (factor Xa, thrombokinase,<sup>334</sup> product I, autoprothrombin C) is a relatively stable proteolytic enzyme, with a molecular weight of 44,000, that has been extensively studied in purified form.<sup>413a</sup> It contains a reactive serine moiety, splits synthetic esters, and activates trypsinogen.<sup>141</sup> Factor X also is "autocatalytically" activated upon storage, and in 25% sodium citrate.<sup>141,279</sup>

The interaction of factor Xa with factor V, Ca<sup>++</sup>, and PF-3 leads to the activation of prothrombin. There is much to favor the view that this reaction (reaction 7) involves the formation of a protein-phospholipid complex<sup>387</sup> termed *prothrombinase* (product II, prothrombin activator of the intrinsic or extrinsic system) in much the same manner as that formed from factor IXa, factor VIII, and PF-3 in the intrinsic pathway (reaction 4).<sup>194,200</sup> Prothrombinase is a particulate substance, and stable preparations of great potency have been isolated by ultracentrifugation. Such preparations may be dissociated, with recovery of activity corresponding to the original three components.<sup>47,241,251</sup> The kinetics of reaction 7 also are consistent with complex formation.<sup>198</sup> Factor Xa apparently

does not lead to the transformation of factor V into an active form,<sup>241</sup> as proposed in the original cascade hypothesis, but reaction 7 is accelerated by the action of traces of thrombin upon factor V (page 429).

There are several plausible alternatives to the hypothesis described above. Thus, purified preparations of factor Xa produce slow activation of prothrombin in the absence of calcium ions or other coagulation factors.<sup>241,336,489</sup> The addition of factor V and PF-3 increases the rate of this reaction<sup>336</sup> and that of esterolytic reactions involving factor X and synthetic substrates.<sup>102</sup> These observations are consistent with the hypothesis that factor V and PF-3 act mainly to accelerate or facilitate the enzymatic action of factor Xa,<sup>137</sup> either by increasing the "susceptibility" of the prothrombin molecule to the proteolytic action of factor Xa,<sup>145,241</sup> or by increasing the affinity of factor Xa for labile bonds in prothrombin.<sup>102</sup>

### Prothrombin Activation

The conversion of prothrombin into thrombin (reaction 8) is calcium-dependent and, when studied in whole blood *in vitro*, is relatively slow but usually complete, i.e., all the prothrombin normally is converted into thrombin.<sup>396,526</sup> The limiting factor appears to be the amount of PF-3 available for reaction 7.<sup>396</sup> Prothrombinase apparently splits the prothrombin molecule in two places,<sup>309</sup> resulting in the transformation of a single polypeptide chain into a molecule composed of two chains of unequal size interconnected by a single disulfide bridge<sup>308,309</sup> (Fig. 10-5, I). This results in the release of peptide material and possibly carbohydrate, a decrease in molecular weight from 69,000 to approximately 34,000, and the evolution of a new terminal amino acid residue.<sup>240,308,309</sup>

### The Seegers Hypothesis

A longstanding and often acrimonious controversy has surrounded the nature of prothrombin and its role in blood coagulation. Over a period of several years, Seegers

and his many collaborators have evolved the hypothesis that the prothrombin molecule is in essence a "molecular system."<sup>450,451</sup> As viewed by these workers, factors VII, IX, and X have no identity as such in plasma, but rather are derived autocatalytically from the parent prothrombin molecule during coagulation.<sup>454</sup> The derivative molecules, which correspond to the activities of factors VII, IX, and X, have been named autoprothrombins I, II, and III; their active forms are designated autoprothrombins A, B, and C. Seegers et al maintain that prothrombin itself is sensitive to surface contact, and dispute the existence of factor XI.

Just as the cascade hypothesis has undergone revision in the past 10 years, the full thrust of Seegers' original hypothesis has been modified in at least three respects (Fig. 10-6): (1) a discrete substance, which gives rise to no other coagulant activity, is now recognized as the immediate precursor of thrombin (prethrombin); (2) the conversion of autoprothrombin III into autoprothrombin C (factor X into factor Xa) is now recognized as a separate and distinct step that occurs prior to "prethrombin" activation; and (3) the role of tissue factor and autoprothrombin I (factor VII, now termed "cothromboplastin") in the activation of autoprothrombin III is now recognized.<sup>33,450,455,457</sup>

The differences between this revised hypothesis and recent versions of the cascade hypothesis (Fig. 10-4) are thus not as great as they once seemed to be.<sup>304</sup> Two major areas of controversy remain. The Seegers hypothesis does not provide an explanation for the role of factor IX in coagulation,<sup>454,456</sup> and maintains the view that the four vitamin K-dependent coagulation factors originate from a single plasma precursor, the "prothrombin complex."<sup>455,457</sup> The evidence cited for the latter premise is derived largely from experiments in which purified prothrombin is slowly activated in 25% sodium citrate.<sup>452</sup> There is now good evidence that prothrombin prepared by methods different from those employed by Seegers<sup>452</sup> does not undergo "autocatalytic" activation in cit-



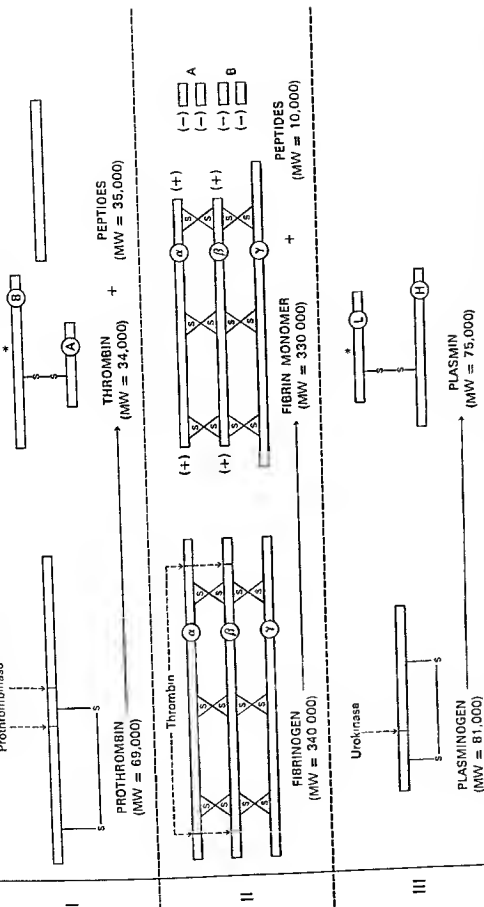


Fig. 10-5. Proteolytic phenomena in blood coagulation. Polypeptide chains of proteins are represented by solid blocks. Asterisks (\*) denote the active centers of thrombin and plasmin. S S denotes disulfide bonds.

I. The activation of prothrombin by prothrombinase. This involves the cleavage of two bonds, yielding peptide material and thrombin, which contains two chains (A and B). The activation of prothrombin by prothrombinase involves the cleavage of two bonds, yielding peptide material and thrombin, which contains two chains (A and B). The activation of prothrombin by prothrombinase involves the cleavage of two bonds, yielding peptide material and thrombin, which contains two chains (A and B).

II. The proteolytic action of thrombin on fibrinogen. This involves the cleavage of four arginyl-glycine bonds and yields fibrin monomer and four small peptides, two from the alpha chains (fibrinopeptides A) and two from the beta chains (fibrinopeptides B). The reduced negative charge on the terminal ends of the alpha end beta chains of fibrin monomer initiates polymerization.

III. The activation of plasminogen by urokinase. This involves the cleavage of a single arginyl-valine bond, yielding a molecule containing a heavy chain (MW = 49,000) and a light chain (MW = 26,000) interconnected by a single disulfide bond. Although activation requires the cleavage of only one bond, there is significant peptide loss, and a second bond presumably is cleaved.

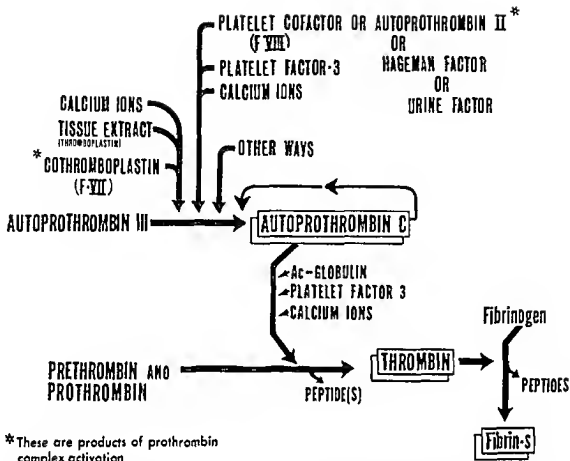


Fig 10-6 Blood clotting mechanisms, according to the Seegers hypothesis. Three basic reactions in blood clotting are represented. Thrombin alone as a proteolytic enzyme produces fibrin. Autoprothrombin C, another proteolytic enzyme, forms thrombin from its precursor. This function of autoprothrombin C is accelerated in a rather specific manner by plasma Ac-globulin, platelet factor 3, and calcium ions. The formation of autoprothrombin C can occur spontaneously, and is accelerated in various ways. Acceleration occurs with calcium ions, tissue thromboplastin, and cothromboplastin (F-VII). The latter group of accelerators tends to lead to complete consumption of autoprothrombin III. Less intensive acceleration is obtained with calcium ions and platelet factor 3 alone or with platelet factor 3, calcium ions, plus platelet cofactor (F-VIII) or instead of platelet cofactor, another platelet cofactor called autoprothrombin II (F-XII) or, in like manner, Hageman protein (F-XIII) or a fraction from urine. There are other ways to form autoprothrombin C. Furthermore, the supplementation of platelet factor 3 activity by any one of the platelet cofactors neither requires nor excludes a preferred sequence in which the platelet cofactor activity occurs (Diagram kindly provided by Dr. Walter Seegers).

rate and that the latter phenomenon, when observed, is a consequence of autocatalytic activation of contaminating factor X.<sup>236,279,308</sup> Furthermore, much of the biochemical data cited elsewhere in this chapter, a large body of immunologic evidence,<sup>110,247</sup> and numerous studies of the hereditary coagulation disorders (Chapter 37) suggest that prothrombin and factors VII, IX, and X are different proteins. Although these data are very difficult to reconcile with the Seegers

hypothesis, available evidence does not justify dogmatic views on either side of this controversy, which must be regarded as unsettled.<sup>304</sup>

### The Thrombin-Fibrinogen Reaction

The last phase of coagulation involves the conversion of fibrinogen into stabilized fibrin, and occurs in three distinct steps<sup>271,462</sup> (Fig. 10-4).

### The Enzymatic Step

Thrombin is a potent proteolytic enzyme that contains a reactive serine moiety at its active center.<sup>167</sup> The molecular weight of purified human thrombin is approximately 34,000.<sup>308,309</sup> Active components with monomeric molecular weights as low as 5,000 have been isolated from bovine thrombin.<sup>48,183,438</sup> Under physiologic conditions, the proteolytic action of thrombin is limited to the cleavage of four arginyl-glycine bonds in fibrinogen<sup>271,462</sup> (Fig. 10-5, II). Thrombin also splits this bond in synthetic esters,<sup>462</sup> but not in many other proteins.<sup>37</sup> This limited specificity appears to be conferred by the location of the enzyme-binding sites on fibrinogen,<sup>71,344</sup> although secondary binding sites on the enzyme remote from its active center may be important.<sup>290</sup> The binding sites on thrombin may be altered without affecting the active center of the enzyme, eg, by acetylation, which abolishes the coagulant activity of thrombin but preserves its esterolytic activity.<sup>275</sup>

The proteolytic action of thrombin on fibrinogen occurs normally in the absence of  $\text{Ca}^{++}$ , and releases four fibrinopeptides per mole of fibrinogen (two each of peptides A and B) (Fig. 10-4, reaction 9). The residual molecule is termed *fibrin monomer*. There is no substantial evidence that carbohydrate moieties are removed from fibrinogen during coagulation.<sup>131,414</sup>

### The Polymerization Step

Because of the loss of acidic peptides, the electronegativity of fibrin monomer is diminished significantly, and the intermolecular repulsive forces between fibrin monomers are diminished. Hydrogen bond donors and acceptors also may be exposed as the result of the removal of the fibrinopeptides, but changes in the conformation of the fibrinogen molecule do not occur.<sup>290</sup> As a result of these processes, polymerization begins. Fibrin polymerization is a complicated physical phenomenon that is still poorly understood.<sup>131,262a,268,462</sup> It apparently involves a

marked change in the ionization of amino acid side chains, and is associated with significant heat production.<sup>134</sup>

Polymerization first leads to the formation of *soluble fibrin* (reaction 10), a term that refers to fibrin formed prior to the action of factor XIII, or in purified systems lacking this proenzyme. This process is reversible *in vitro*, and involves primarily the formation of hydrogen bonds between fibrin monomers.<sup>134,462</sup> As a consequence, soluble fibrin is mechanically fragile, and dissociates into its constituent monomers in the presence of inhibitors of hydrogen bonding, such as urea or monochloroacetic acid.<sup>130,507</sup>

### The Stabilization Step

In the stabilization step, the last in the thrombin-fibrinogen reaction, soluble fibrin is converted into *insoluble* or *stabilized fibrin* by factor XIIIa (reaction 12).<sup>288,289,293</sup> This enzyme is formed from its inert precursor by the action of thrombin (reaction 11). This involves removal of a peptide of MW 4,000 from the alpha chain of the proenzyme.<sup>447</sup> Factor XIIIa ("fibrinolygase") is a transamidase that forms covalent bonds between the epsilon amino groups of lysine and the gamma amide groups of glutamine.<sup>294,460</sup> The transamidation step requires calcium, but the activation of human factor XIII by thrombin apparently does not.<sup>508</sup>

Under the electron microscope,<sup>183</sup> stabilized fibrin reveals a characteristic axial periodicity of 226 nm, which is not present in soluble fibrin. This may be due to rearrangement during stabilization of fibrin monomers in a "staggered" side-by-side manner.<sup>40</sup> Structurally, fibrin resembles the proteins of muscle and skin. Insoluble fibrin provides an extremely strong and stable framework for the "permanent" hemostatic plug.

### Miscellaneous Coagulation Phenomena

#### Autocatalytic Action of Thrombin

The rate of prothrombinase production via the intrinsic pathway is initially relatively

slow. However, once traces of thrombin evolve, the rate of these reactions increases greatly, ie, the production of prothrombinase and hence of thrombin becomes "autocatalytic." This phenomenon has been recognized for many years<sup>502,527</sup> and is now thought to be the result of an interaction between thrombin and factors V and VIII (*reaction 13*, Fig. 10-4).<sup>64,212,251,416</sup> Traces of thrombin increase the activity of factor VIII by 80-fold.<sup>416</sup> The activity of factor V increases 15- to 30-fold, but more thrombin is required.<sup>212</sup> High concentrations of thrombin inactivate both factors

The autocatalytic role of thrombin has been viewed as the process that "shifts coagulation into high gear."<sup>416</sup> This may be homeostatically important, since the rapid evolution of a massive coagulum may be essential for hemostasis following large injuries

### Role of Metal Ions

Calcium ions are required for all reactions in the coagulation phase except those of the contact phase involving factors XII and XI (Fig. 10-4, *reactions 1 and 2*), and the enzymatic effects of thrombin on fibrinogen (*reaction 9*) and factor XIII (*reaction 12*). Calcium ions apparently act as nonspecific accelerators of fibrin polymerization, but are not an absolute requirement for this step (*reaction 10*).<sup>425</sup>

The exact biochemical role of this divalent ion in these diverse reactions remains obscure. There is indirect evidence that calcium functions in an adsorbed rather than in an ionic form, where it may act to maintain optimal surface charge, or to stabilize the subunit structure or conformation of procoagulant proteins, phospholipids, or complexes thereof.<sup>294,402</sup>

The removal of trace metal ions by strong chelating agents such as EDTA markedly alters the in vitro stability of factors V and VIII<sup>533</sup> and possibly fibrinogen as well.<sup>169,549</sup> There is no evidence, however, that any of these factors are true metalloproteins. The marked impairment of fibrinogen reactivity produced by EDTA apparently is not the

result of the removal of essential trace ions.<sup>67,169</sup> The rapid in vitro inactivation of factors V and VIII by EDTA is poorly understood.<sup>533,549</sup>

### Consumption of Coagulation Factors

The coagulation factors have long been separated into two groups on the basis of their presence or absence in serum (Table 10-2), ie, those that are utilized or consumed during in vitro coagulation (fibrinogen, prothrombin, factors V, VIII, and XIII), and those that are not (factors VII, IX, X, XI, and XII). At least three different phenomena may be involved in the in vitro "consumption" of coagulation factors: (1) the factors may be converted stoichiometrically into their "active" forms, eg, fibrinogen and prothrombin; (2) the active forms may be adsorbed into fibrin and thus removed from serum with the clot, eg, factor XIII<sup>202</sup>; and (3) the factors may first be activated by thrombin, as discussed above, and then consumed in an undefined manner, eg, factors V and VIII.

When unphysiologic concentrations of activators are employed, factors that normally remain in serum are consumed.<sup>368</sup> For example, factor X is completely utilized when excess factor VIII is present<sup>306</sup>; factor IX is consumed in the presence of large concentrations of Celite.<sup>365,523</sup> Serum free of all coagulation factors has been prepared by such methods. Conversely, when certain factors are deficient, substances normally "consumed" are not utilized,<sup>458</sup> ie, factor V consumption is reduced in blood deficient in factors VIII and IX.<sup>126</sup> This phenomenon provides the basis for the prothrombin consumption test (page 1059).

## Homeostatic Control Mechanisms

As has been discussed, the proenzymes involved in the process of coagulation are present in great excess, minute concentrations of coagulant enzymes activate large amounts of their substrates, and, once initiated, coagulation becomes autocatalytic. Because these

phenomena lead to biologic "amplification" of the processes of coagulation, counterforces of equal potency are needed to limit the hemostatic plug to desirable size and to neutralize active procoagulants that may enter the general circulation. Such control mechanisms operate at three different physiologic "levels," ie, locally in the hemostatic plug, in the general circulation, and in various cellular clearance mechanisms residing in the reticuloendothelial system, the liver, and the lung.

### Local Processes

Blood flow, per se, acts to restrict the propagation of blood clots; active coagulants are washed away from the site of injury and are greatly diluted in the process. Fibrin forms a firm seal over injured vascular surfaces and contiguous normal endothelium, thus restricting active coagulants to the interior of the hemostatic plug. The role of the platelet in limiting the extension of the hemostatic plug is discussed on page 399.

The confusing term "antithrombin I" refers to the capacity of fibrin to adsorb or occlude thrombin.<sup>257,413</sup> This phenomenon is a unique form of product inhibition of an enzymatic reaction, and may neutralize a surprisingly large amount of thrombin. For example, as many as 1000 units of thrombin can be adsorbed by the fibrin formed from 1 ml of plasma. The thrombin so adsorbed is not inactivated; it can be recovered, essentially unaltered, by extraction of the clot, or may reappear slowly during clot retraction<sup>204</sup> or fibrinolysis.<sup>449</sup> The ability of a fibrin mass to rapidly and instantaneously adsorb large amounts of thrombin at the site of its production suggests that "antithrombin I" may be of great physiologic significance.

### Humoral Inhibitors

Several substances that act as inhibitors of coagulation *in vitro* have been demonstrated in normal plasma. The *in vivo* action and the homeostatic importance of such humoral inhibitors of coagulation remain unclear.<sup>204</sup>

### Antithrombins

Six antithrombins have been designated with Roman numerals, but it has become apparent that only "antithrombin I," which is not a humoral inhibitor and is discussed above, and antithrombin III are clearly of physiologic importance. Antithrombin II is the heparin cofactor, which is discussed on page 1239; antithrombin VI represents the effects of various fibrin degradation products (FDP), which are discussed on page 436. Antithrombins IV<sup>338</sup> and V<sup>318</sup> are of uncertain physiologic significance.

### Antithrombin III

Antithrombin III is a plasma protein that inactivates thrombin by means of an irreversible time-dependent reaction, and thus is often designated "progressive" antithrombin. It has been purified extensively,<sup>6,204</sup> and has been separated from two other "progressive" antithrombins,<sup>9</sup> which are discussed below. Antithrombin III is an alpha-2 globulin that has a molecular weight of 64,000.<sup>7</sup> It apparently is produced in the liver, and is found in various extravascular sites as well as in the plasma.<sup>204</sup>

The reaction between antithrombin III and thrombin has been carefully studied,<sup>172,205,358</sup> but the biochemical mechanism of thrombin inactivation remains obscure. Kinetic studies suggest that the reaction is bimolecular,<sup>7,204</sup> but unexplained inconsistencies in the stoichiometry have been encountered.<sup>257</sup>

Antithrombin III inactivates, in addition to thrombin, factor Xa,<sup>65,314,545</sup> factor VII,<sup>133</sup> plasmin, and possibly tissue factor<sup>133</sup>; its action is inhibited by platelet factor 2 (page 397). Most evidence would suggest that this inhibitor is identical or closely related to antithrombin II,<sup>9</sup> although this view has been questioned.<sup>160</sup>

### Miscellaneous Antithrombins

A second "progressive" antithrombin normally present in plasma is an alpha-2-macroglobulin.<sup>8,9</sup> This protein acts more slowly than antithrombin III, forms complexes with

of proteolytic enzymes other than thrombin, and inactivates plasmin<sup>161</sup> (page 435). A third progressive antithrombin (*alpha-1-trypsin inhibitor*) is normally present in plasma. Fibrinopeptides may act as antithrombins.<sup>189</sup>

### Miscellaneous Coagulation Inhibitors

Two inhibitors of factor XIa (contact product) have been isolated from normal plasma. One is an alpha globulin and has a molecular weight of 40,000 to 50,000<sup>354,370</sup>; preliminary studies suggest that this substance also inactivates factor Xa.<sup>143</sup> The other is an inhibitor of the first component of complement.<sup>44</sup>

Other "physiologic" inhibitors of coagulation include substances in the plasma that inactivate factor IXa,<sup>133</sup> prothrombinase,<sup>115</sup> and tissue thromboplastin.<sup>273</sup> Various lipid inhibitors have been isolated from normal blood and tissues,<sup>250</sup> including some that may participate in the inactivation of factor VIII.<sup>173,311</sup> The role of humoral inhibitors in the pathogenesis of hemophilia A is discussed on page 1161.

### Cellular Clearance Mechanisms

The removal of activated coagulants from the circulation by the liver has been demonstrated both directly and indirectly.<sup>116,483</sup> The reticuloendothelial depots in this organ<sup>26</sup> remove mainly particulate material, eg, prothrombinase,<sup>484</sup> tissue thromboplastin, certain fibrinogen degradation products.<sup>163</sup> Soluble coagulants, such as the stable enzymes, that are present in serum in high concentrations (factor IXa, factor Xa, factor VII<sup>117,479,481</sup>), also are cleared from the circulation by the liver, but appear to be taken up by the hepatic cells, possibly by means of conjugation with cell-associated inhibitors.<sup>118</sup> All of these factors are glycoproteins, and it is not improbable that, as with many other plasma proteins,<sup>339</sup> the loss of terminal sialic acid moieties during activation results in their removal from the circulation.<sup>117,536</sup>

Finely particulate fibrin<sup>281</sup> and tissue fac-

tor<sup>32</sup> may be removed from the circulation in the *pulmonary vascular bed*. Circulating *leukocytes* also may participate in the clearance of hemostatic "debris."

The "autoblockade" of these clearance functions is an essential part of the Shwartzman phenomenon in animals, and may be of pathophysiologic importance in intravascular coagulation in man (page 1211).

## The Fibrinolytic Enzyme System

Fibrinolysis results from the conversion of an inert plasma proenzyme (plasminogen) into a proteolytic enzyme (plasmin) whose main physiologic role presumably is the proteolytic dissolution of fibrin. Plasminogen and plasmin, together with activators and inhibitors of the process, comprise the fibrinolytic enzyme system (Fig. 10-7). Fibrinolysis is usually considered to be the major physiologic means of disposing of fibrin after its hemostatic function has been fulfilled; it would thus be of paramount importance in wound healing and the recanalization of thrombosed vessels.<sup>266</sup> However, other mechanisms of fibrin removal, such as phagocytosis, may be of equal importance.<sup>41,285</sup> Knowledge concerning fibrinolysis is now extensive<sup>143,151,358,391,467</sup> but, like coagulation, it has been studied largely by *in vitro* techniques. The details of the process as it occurs *in vivo* are only now becoming clarified.

- The therapeutic activation of the fibrinolytic enzyme system (thrombolytic therapy) and abnormalities of the system that may be of pathophysiologic importance in thrombosis are discussed in Chapter 39. The pathophysiologic role of fibrinolysis and fibrinogenolysis in various bleeding disorders is summarized in Chapter 38.

### Components of the System

#### Plasminogen

Plasminogen is a beta globulin, of molecular weight 81,000, which is composed of a

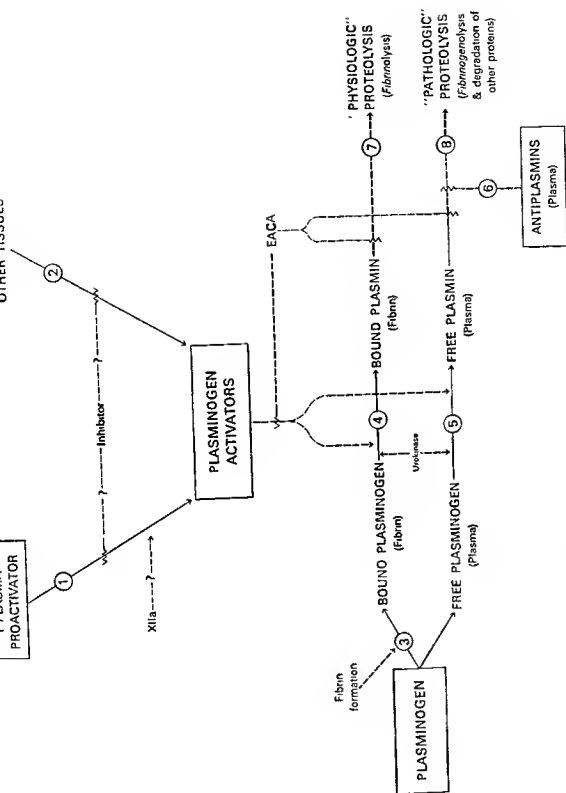


Fig 10.7 The physiology of fibrinolysis (essentially as proposed by Sherry<sup>415</sup>). Solid arrows denote transformation; dashed arrows denote action. The steps numbered within arrows are referred to in the text. The physiologic importance of steps or components designated with question marks has not been definitely established. EACA is an abbreviation for epsilon amino-caproic acid.

single polypeptide chain.<sup>2,17,435,496</sup> It is present in the plasma in concentrations ranging from 10 to 20 mg/dl.<sup>465</sup> Evidence that it is synthesized in the liver is largely indirect, and there is preliminary evidence that it is produced or stored in the eosinophils of the bone marrow, and may be transported in the circulation within these cells.<sup>43,356,408,431</sup>

Plasminogen forms complexes with fibrinogen,<sup>39</sup> and, when fibrin forms, large amounts of plasminogen are adsorbed or occluded within the fibrin mass.<sup>430</sup>

### Plasmin

The biochemical properties of plasmin (fibrinolysin) vary, depending on the means by which this substance is activated.<sup>19,496</sup> Urokinase activation involves the cleavage of a single arginyl-valine bond<sup>435</sup> and the conversion of a long polypeptide chain into a molecule composed of two chains<sup>436,495,496</sup> held together by a single disulfide bond (Fig. 10-5, III).

Unlike thrombin, plasmin has a wide spectrum of proteolytic activity that is quite comparable to that of trypsin.<sup>467</sup> It cleaves arginyl-lysine bonds in a large variety of substrates including synthetic esters, hormones, various components of complement,<sup>391,467</sup> and several coagulation factors including fibrinogen<sup>123,477</sup> (page 1225).

Plasmin is very short-lived in plasma, owing to its inactivation by humoral antiplasmins. There is preliminary evidence that a complex composed of plasmin and antiplasmin of the alpha-2 macroglobulin variety may normally circulate in the plasma.<sup>188</sup>

### Plasminogen Activators

The term "plasminogen activators" refers to a heterogeneous group of substances that convert plasminogen into plasmin. Such plasminogen activators apparently are proteolytic enzymes,<sup>469</sup> but their biochemistry is poorly understood. They are concentrated in the lysosomes of most cells<sup>345</sup> and in the vascular endothelium<sup>16,505</sup> (*tissue activators*, *fibrinolytic enzymes*, *cytokinases*). The concentration

of endothelial activators is greatest in the vessels of the microcirculation, particularly in small veins and in the renal vasculature.<sup>223,323</sup> Relatively little plasminogen activator is found in large vessels. Plasminogen activators also have been isolated from leukocytes<sup>114</sup> and platelets,<sup>171,177</sup> and there is preliminary evidence that thrombin may activate plasminogen directly.<sup>83,135</sup>

Plasminogen activators normally are present in trace amounts in the plasma. There is indirect evidence that such *blood activators* originate from the endothelium, and enter the general circulation where they survive only transiently because of rapid removal by the liver.<sup>153</sup>

*Urokinase* is a plasminogen activator that has been isolated from urine and extensively purified.<sup>281</sup> It is a polypeptide, of molecular weight 54,000,<sup>281</sup> that cleaves arginyl-lysine bonds in plasminogen and in a variety of other substrates. Urokinase apparently is not, as previously supposed, an excretion product derived from plasminogen activators in the blood.<sup>222,263</sup> There is preliminary evidence that it is synthesized in the kidney.<sup>263</sup>

Plasminogen activators also are present in other body fluids, eg, milk,<sup>33</sup> tears,<sup>491</sup> saliva,<sup>15</sup> and semen.<sup>510</sup> These activators, and urokinase as well, may play a physiologic role in maintaining the patency of excretory ducts.<sup>328</sup>

### Plasminogen Proactivator

Evidence for the existence of an inert precursor of blood plasminogen activator (*plasma proactivator*) was first obtained from studies of plasminogen activation by streptokinase. These studies suggested that this bacterial enzyme activated plasminogen only after a preliminary interaction with an unidentified plasma euglobulin<sup>258,467</sup> (Fig. 10-7, *step 1*). A further study of this phenomenon, however, provided an alternative explanation for the data; ie, the plasma factor involved was plasmin, which, in the form of a complex with streptokinase, is capable of autocatalytically activating more plasminogen.<sup>258</sup> This hypothesis, in turn, has been criticized,<sup>469</sup> and



the mechanism of plasminogen activation by streptokinase remains controversial.<sup>112,259,497</sup>

Supporting evidence for the presence of a plasma proactivator has been obtained from studies demonstrating the ability of factor XIIa to activate fibrinolysis.<sup>230,353</sup> In vitro studies with purified factor XII suggest that plasminogen activation occurs only in the presence of one or possibly two plasma proteins that are distinct from factor XI and plasma kallikrein.<sup>252,377</sup> In vivo studies in patients with factor XII deficiency provide indirect support for this hypothesis.<sup>320,321,232</sup> Fibrinolysis, like coagulation, may thus be activated by both extrinsic (tissue-activated) and intrinsic (contact-activated) pathways.

## Inhibitors of Fibrinolysis

### Antiplasmins

At least two plasma proteins specifically neutralize free plasmin.<sup>362,363</sup> These are an alpha-2-macroglobulin, which neutralizes plasmin rapidly, and a slowly acting alpha-1 globulin (alpha-1-antitrypsin).<sup>432</sup> The alpha-2-macroglobulin is a competitive inhibitor of both thrombin and plasmin, and competition between these two enzymes for binding sites on the inhibitor molecule may be homeostatically important in intravascular coagulation<sup>161</sup> (Chapter 38).

The kinetics and stoichiometry of the plasmin-antiplasmin reaction have been studied intensively. In plasma, the total antiplasmin activity normally exceeds the available plasmin by at least 10-fold.<sup>363</sup> There is indirect evidence that antiplasmins are synthesized in the liver,<sup>511</sup> but data concerning their biodynamics are inadequate.

Antiplasmins also have been isolated from the platelets,<sup>243</sup> and from mesothelium and endothelium<sup>411</sup>; the physiologic significance of these tissue-associated antiplasmins remains uncertain. Various plasma lipids exhibit antiplasmin activity in vivo and in vitro, an observation of possible physiologic and pathologic significance.<sup>129,331</sup>

Natural inhibitors of plasminogen activa-

tion also have been demonstrated in the plasma.<sup>53</sup>

### Artificial Inhibitors

A number of chemical agents have been shown to inhibit fibrinolysis.<sup>165,343</sup> Some of these have found therapeutic utility in man, eg, epsilon aminocaproic acid [EACA],<sup>20</sup> tranexamic acid,<sup>127</sup> Trasylol.<sup>129</sup> These are discussed briefly on page 1226.

## The Physiology of Fibrinolysis

### Release of Activator

Most of the physiologic and pathologic stimuli that lead to the release of plasminogen activators in vivo are vasoactive<sup>105,145,218</sup> and presumably lead to the release of endothelial activators (Fig. 10-7, step 2), eg, exercise,<sup>91</sup> electric shock and other forms of stress,<sup>440</sup> epinephrine, histamine,<sup>219</sup> bacterial pyrogen,<sup>144</sup> intravenous nicotinic acid,<sup>153</sup> ischemia, and hypoxia.<sup>267,440</sup> Lysosomal activators also may be released under physiologic circumstances,<sup>50</sup> and in pathologic processes involving shock or tissue damage. The physiologic importance of plasminogen activated "indirectly" by factor XIIa, or directly by thrombin, is uncertain. Fibrinolytic activity normally is much greater in the microcirculation than in the general circulation,<sup>223</sup> presumably because of the release of endothelial activators.

### Plasminogen Activation

The consequences of plasminogen activation are greatly modified by the tendency of fibrin to adsorb this proenzyme.<sup>20,467</sup> Thus, when fibrin forms (Fig. 10-7, step 3) and plasminogen is activated (steps 4 and 5), plasmin exists in both free and fibrin-adsorbed forms. Free plasmin normally is destroyed as rapidly as it is formed by antiplasmins in the plasma (step 6), and hence is unable to proteolytically degrade any of its many susceptible substrates. Fibrin-bound plasmin, to the contrary, is little affected by the antiplasmins

in the plasma, and thus is free to carry out its presumed physiologic function, the lysis of fibrin (step 7). The fact that antiplasmins are ineffective within a fibrin thrombus is unexplained, although there is some evidence that these inhibitors are unable to diffuse into a fibrin mass as rapidly as can plasminogen activators.<sup>542</sup>

The proteolytic degradation of fibrinogen, other coagulation factors, and plasma proteins (step 8) occurs only if free plasmin exceeds the capacity of its plasma inhibitors.<sup>477</sup> This is a pathologic process (*fibrinogenolysis*, pathologic proteolysis); it is discussed in Chapter 38. Fibrinolysis is thus regulated mainly by the levels of available plasminogen activators. Although plasminogen may be activated in the general circulation, the proteolytic action of plasmin is restricted to the site where it is homeostatically required, ie, in a fibrin mass. An alternative explanation for *in vivo* fibrinolysis is consistent with most of the available data, ie, plasmin forms inactive complexes with its inhibitors, which dissociate when they are adsorbed by fibrin.<sup>35</sup>

The *in vivo* activity of the fibrinolytic enzyme system is modified by several additional factors. Thus, diffusion of plasminogen activators into preformed thrombi may be relatively slow,<sup>376</sup> and fibrinolysis appears to be relatively slow in the general circulation. Such observations would suggest that this enzyme system plays only a minor role in lysing large thrombi in major vessels. However, when fibrin formation occurs in the presence of activator, activator is adsorbed and incorporated into the fibrin as it forms, and fibrinolysis may be very rapid. In intravascular coagulation, for example, massive amounts of fibrin may be lysed within a matter of minutes (Chapter 38). This phenomenon appears to be particularly important in the microcirculation, owing to the normal presence of high concentrations of activator. The action of factor XIII increases the resistance of fibrin to the proteolytic action of plasmin,<sup>171,201,291</sup> but the physiologic importance of this phenomenon is uncertain.

## Physiologic Variations

There is preliminary evidence that fibrinolysis follows a circadian rhythm, and is normally greater during the nocturnal hours.<sup>147</sup> Fibrinolysis also is more active in the elderly<sup>408</sup> and in females,<sup>91</sup> but is diminished in obese persons<sup>375</sup>; it is unaffected by the menses.<sup>84</sup> In pregnancy, fibrinolytic activity is subnormal at term, but rises rapidly to supernormal levels after delivery<sup>85,460</sup>; the levels of plasminogen closely parallel those of fibrinogen.<sup>81,219</sup> Anovulatory medications apparently do not significantly affect the fibrinolytic enzyme system.<sup>65</sup> A mild deficiency of plasminogen is present in normal neonates,<sup>52</sup> and in hypometabolic states<sup>51</sup>; high levels are present in hypermetabolism.<sup>54</sup>

## The Proteolytic Degradation of Fibrin: Degradation Products

The proteolytic action of plasmin on fibrin or fibrinogen leads to the formation of a family of soluble protein fragments (degradation or split products, FDP, FSP).<sup>265,517</sup> Intensive study of this process has provided convincing evidence that the sequence of events to be described below occurs physiologically, and proceeds in essentially the same manner whether *fibrinolysis* or *fibrinogenolysis* is involved. The major antigenic determinants of native fibrinogen are retained in fibrin and in the larger degradation products.<sup>315</sup> The immunologic methods usually used for the assay of these fragments do not distinguish between degradation products of various sizes, or between fibrin degradation products and those derived from fibrinogen.<sup>403a</sup> Consequently, throughout this book the abbreviation "FDP" will be used with reference to all of these substances.

The degradation of fibrin is a stepwise process (Fig. 10-8), and the molecular size of the resulting FDP thus varies with the duration of plasmin action. In the initial step, approximately 20% of the fibrinogen molecule is removed in the form of small peptides,

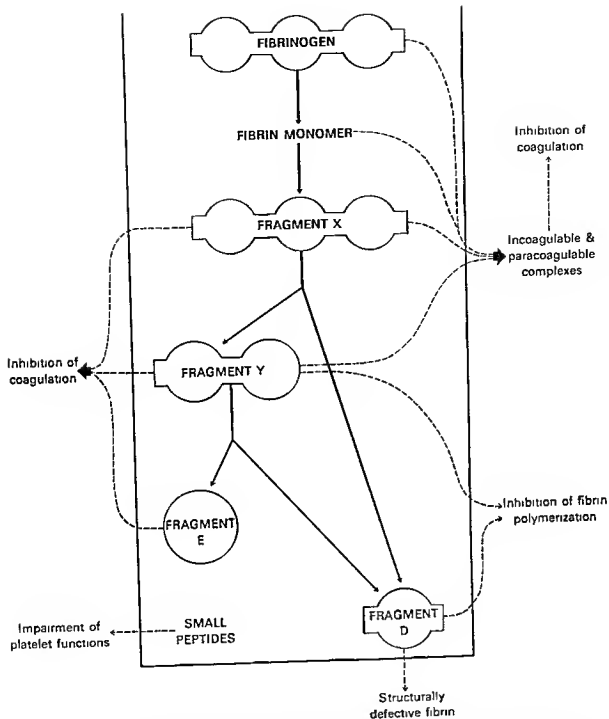


Fig 10-8. The proteolytic degradation of fibrinogen or fibrin the biologic effects of degradation products. The stepwise degradation of the fibrinogen or fibrin molecule by plasmin, essentially as viewed by Marder,<sup>315</sup> is indicated in the center block. Effects of various FDP on hemostatic functions are indicated by dashed lines. The symbols represent the trinodular model of fibrinogen structure, proposed by Hall and Slaytor.<sup>193</sup> The E fragments may represent the N-terminal disulfide knots.<sup>315</sup>

yielding *fragment X* (first derivative).<sup>151</sup> This fragment has a molecular weight of approximately 280,000, retains thrombin binding sites, and resembles but is not identical to<sup>333</sup> the soluble fibrinogen derivatives (fraction I-8) that normally are present in plasma.<sup>463</sup> The proteolysis of fragment X may yield a variety of large FDP, ranging in molecular weight from 100,000 to 200,000 (intermediate fragments)<sup>151,151</sup> Alternatively, this fragment may be split into one molecule of fragment D and one of fragment Y.<sup>315,317</sup> *Fragment Y* has an estimated molecular weight of 155,000. It is split into one molecule of fragment D and one of fragment E. The molecular weight of *fragment D* is approximately 90,000, while that of the smaller *fragment E* ranges from 30,000 to 50,000. There is preliminary evidence that fragment D is derived from the lateral nodules of the fibrinogen molecule (Fig 10-8), and that fragment E represents the "disulfide knot" of the central nodule (Fig. 10-1).<sup>315</sup> Both fragmented D and E are relatively resistant to further proteolysis by plasmin.

The larger fragments X and Y form soluble complexes with fibrinogen and fibrin monomer.<sup>462,474</sup> These complexes dissociate in the presence of alcohol and protamine sulfate to form gels and precipitates of various types, a phenomenon that has been termed *paracoagulation*.<sup>86,178,448</sup> Such complexes precipitate in the cold and in heparinized plasma (*cryofibrinogens*).<sup>340,341</sup>

### Biologic Effects of FDP

FDP and complexes thereof profoundly impair the hemostatic process, and are a major cause of hemorrhage in intravascular coagulation and fibrinolysis (Chapter 38). Extant assay methods determine only the total amount of FDP present, and evidence concerning the effects produced by each particular fragment is incomplete. Most FDP are inhibitors of coagulation, *fragment Y being the most potent in this respect.*<sup>152,466</sup> They are potent antithrombins (antithrombin VI), and also form incoagulable or slowly coagulating complexes with fibrin monomer or

fibrinogen. Poorly defined effects on other steps in coagulation have been demonstrated.<sup>357,506</sup> Fragments Y and D inhibit fibrin polymerization.<sup>21</sup> Fragment D, when incorporated into fibrin, produces a structurally defective fibrin polymer.<sup>151</sup> Purified fragment E is a potent inhibitor of thrombin.<sup>277</sup>

Various products of fibrinogen degradation impair platelet functions, but the details of this process are poorly understood (page 432).<sup>475</sup> Preliminary data would suggest that inhibition of ADP-induced platelet aggregation is due mainly to the effects of small dialyzable peptides.<sup>483,503</sup> Such small peptides also may be vasoactive.<sup>262</sup> Fibrin monomers and complexes thereof also may produce "hyperaggregability" of platelets, which may lead to in vivo clumping and sequestration.<sup>44,503</sup> "Paracoagulable" complexes also inhibit coagulation.<sup>262</sup>

FDP are removed from the circulation by clearance mechanisms in the liver and reticuloendothelial system.<sup>163</sup> The half-life of these fragments, as a group, is approximately nine hours.<sup>152</sup>

## Homeostatic Significance of Coagulation

### Hemostasis

Evidence obtained from in vitro experiments, as discussed above, suggests that the intrinsic and extrinsic pathways provide alternative methods of activating factor X. However, the presence of severe bleeding in patients with factor VII deficiency (Chapter 37) suggests that the extrinsic as well as the intrinsic pathway is essential for normal hemostasis. The physiologic role of the extrinsic pathway is, however, uniquely difficult to study. Animals with hereditary factor VII deficiency form hemostatic plugs normally, and do not provide a valid model since the canine disease is not associated with significant bleeding.<sup>227</sup> The major physiologic importance of the extrinsic pathway may lie in its ability to initiate the rapid evolution of

the first traces of thrombin and thus to initiate the autocatalytic phase of coagulation by activating factors V and VIII. Traces of thrombin also may further platelet aggregation (page 393) and lead to the formation of small amounts of fibrin.

Deficiency of factor XII (Hageman factor) is not associated with a significant hemorrhagic diathesis, despite the presence of gross abnormalities in various *in vitro* tests of blood coagulation (page 1179). This inconsistency would seem to raise serious questions concerning the physiologic importance of factor XII in the intrinsic pathway. Several ingenious experiments of questionable physiologic significance<sup>60</sup> suggest that, in factor XII deficiency, the intrinsic pathway may be activated by alternative mechanisms, ie, an interaction between factors VIII and IXa and substances derived from the platelets,<sup>45a</sup> or with the tissue factor-factor VII complex formed in the extrinsic pathway.<sup>60,63,365</sup> Such an alternate pathway could not activate factor X in the absence of factors VIII and IX, but could "bypass" factors XII and XI.<sup>60</sup> This hypothesis does not, however, explain why patients with factor XI deficiency bleed abnormally.<sup>369</sup>

The study of experimental wounds in animals with hereditary coagulation disorders<sup>227</sup> provides good evidence that the major hemostatic role of fibrin formation is to consolidate the temporary platelet thrombus, as discussed in Chapter 9. Thus, initial hemostasis was normal in factor IX-deficient dogs, but was delayed in factor VIII-deficient animals. In both groups, the hemostatic plug was deficient in fibrin and was loose and friable; re-bleeding was usually seen.<sup>227</sup> The observed differences between factor VIII- and factor IX-deficient animals remain unexplained, as does the fact that patients with hereditary afibrinogenemia do not bleed as severely as those with hemophilia A.

The unanswered questions discussed above emphasize an important axiom, ie, inferences drawn from the *in vitro* study of blood coagulation may have little, if anything, to do with the process as it occurs *in vivo*. The hemostatic role of coagulation may be even

more complex and much more subtle than is presently realized.

### The Question of Physiologic Intravascular Coagulation

It has long been theorized that the hemostatic process plays a continuous role of physiologic importance, in addition to its intermittent activity when vessels are injured. In terms of this hypothesis, platelets are continuously required for endothelial support, and a film of fibrin is continuously deposited on the endothelial surface of normal vessels, as a result of "physiologic intravascular coagulation,"<sup>107</sup> and is continuously removed by means of "physiologic fibrinolysis."<sup>1325</sup> This cycle is viewed as essential in maintaining normal vascular integrity, and the resulting utilization of platelets and coagulation factors is considered to be a major determinant of their *in vivo* catabolism.<sup>89,271,481</sup>

There is now considerable evidence against this hypothesis, however satisfying it may be teleologically.<sup>213</sup> The coagulation factors are catabolized more rapidly than other plasma proteins but available evidence would suggest that this is not a consequence of their continuous "physiologic consumption." Thus, the *in vivo* turnover rate of fibrinogen is unaffected by severe deficiencies of various coagulation factors, heparin and coumarin anticoagulants, or fibrinolytic enzyme inhibitors such as EACA,<sup>129</sup> all of which would be expected to retard cyclic intravascular coagulation and fibrinolysis. Studies of the effects of the above factors on vascular permeability or fragility have yielded largely inconclusive evidence. Electron microscopy has failed to demonstrate a fibrin film on normal endothelium, and the evidence for an "endothelial supporting function of platelets," discussed at length on page 399, remains unconvincing.<sup>213</sup>

The evidence cited above leads to the following conclusions: (1) coagulation and fibrinolysis are undoubtedly involved in the repair of numerous small or imperceptible vascular injuries that occur daily under normal circumstances; (2) even though this

process is essentially continuous, it does not appear to operate on normal uninjured endothelium; and (3) although small amounts of the various coagulation factors are undoubtedly utilized in this manner, there is no evidence that such "physiologic consumption" or "physiologic fibrinolysis" is a major factor in determining the *in vivo* catabolism of these proteins.

### Non-hemostatic Processes

Blood coagulation factors have been studied almost exclusively in terms of their role in hemostasis and in thrombosis (Chapter 39). Evidence suggesting that coagulation is involved in various other physiologic and pathologic processes is now accumulating.<sup>260,323,420</sup>

### Inflammation

Preliminary evidence would suggest that *factor XIIa* is required for the release of plasma kinins from their precursors in normal plasma,<sup>321</sup> and may activate the kinin system *in vivo*.<sup>369,424</sup> This factor also may be involved in the release of kinins from leukocytes,<sup>329</sup> and in the activation of C'1 esterase.<sup>124</sup> *Plasmin* activates both the kinin

system<sup>356,519</sup> and the early-reacting components of the complement system.<sup>280,399</sup> It also inactivates C'1 esterase inhibitor (page 333).<sup>186</sup>

The studies discussed above have led to the hypothesis that factor XIIa serves as a surface-sensitive "trigger mechanism" that translates the stimuli of injury into diverse homeostatic responses, i.e., hemostasis, immunologic and cellular defense, and repair (Fig. 10-9). However, there is no evidence that factor XII deficiency is associated with abnormal susceptibility to infection, and the role of this factor in inflammation, as in coagulation, remains a scientific curiosity. The activation of the kinin system by factor XIIa may be of pathophysiologic importance in gout, a disorder in which factor XII may be activated by sodium urate deposits in the synovia.<sup>254</sup>

### Wound Healing

Hereditary deficiencies of fibrinogen, factor XIII, and the dysfibrinogenemias (page 1174) are frequently associated with abnormal scar formation, wound dehiscence, post-circumcision bleeding, and bleeding from the umbilical stump. These findings are uncommon in other hereditary coagulation disorders, and have led to the hypothesis that optimal amounts of normal, stabilized fibrin

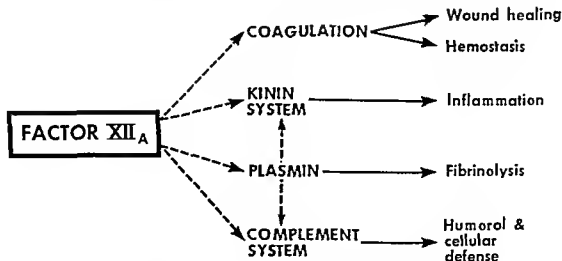


Fig 10-9. The diverse consequences of factor XII activation

are essential for normal wound healing.<sup>131</sup> There is some experimental support for this concept.<sup>51,266</sup> Thus, when fibroblasts are cultured in vitro in human plasma, fibrin appears to orient fibroblastic proliferation, a process that is grossly defective in factor XIII-deficient plasma.<sup>51</sup>

### Tumor Localization

The metastatic spread of neoplasms may depend on the presence or formation of fibrin at the site of lodgement of tumor emboli.<sup>493</sup> This has been attributed to elaboration of a coagulant substance by tumor cells.<sup>493</sup> Anti-coagulant drugs and agents that activate the fibrinolytic enzyme system appear to limit or retard neoplastic spread in some animal tumor systems,<sup>14</sup> but not in others.<sup>182</sup> Evidence concerning human tumors is equivocal.

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## SECTION 5. *Blood Groups and Blood Transfusion*



### *Transfusion of Red Cells*

#### **Blood Groups**

##### **ABO and Lewis Systems**

###### **Immunochemistry**

###### **Distribution of ABH and Lewis Substances**

###### **Ontogeny of ABH Blood Group Substances**

###### **ABO Blood Groups and Human Disease**

##### **II System**

##### **Rh System**

##### **MN, Ss, U System**

##### **P System**

##### **Other Systems: Kell, Kidd, Lutheran, Duffy**

##### **Blood Groups and Human Genetics**

##### **Medicological Applications**

##### **Application to Anthropology—The Ethnologic Distribution of Blood Groups**

##### **Methods of Blood Typing**

##### **Tests of Compatibility**

##### **Collection and Preservation of Blood**

###### **Storage Lesion**

###### **Anticoagulants**

###### **Frozen Red Cells**

##### **Blood Transfusion**

###### **Indications**

###### **Mode and Route of Administration**

###### **Complications of Blood Transfusion**

###### **Immunologically Mediated Transfusion**

###### **Reactions**

###### **Nonimmune Transfusion Reactions**

##### **Clinical use of Plasma and Plasma Derivatives**

##### **Plasma**

##### **Plasma Fractions**

###### **Albumin Preparations**

###### **Immunoglobulins**

###### **Clotting Factors**

###### **Plasma Enzymes and Enzyme Inhibitors**

#### **Blood Groups**

Blood groups represent systems of antigenic determinants found on the surface of red cells. These antigens are inherited according to simple mendelian laws and the major systems (eg, ABO and Rh-Hr) are inherited independently of each other.

Blood groups still are most readily identified by means of specific antibodies present in serum, either "naturally" or after immunization with foreign red cells or soluble blood group substances. The chemical characterization of blood group antigens has lagged far behind serologic and genetic studies and so far only the ABO, Lewis, and, to a lesser extent, the *Ii* and *MN* systems have lent themselves to extensive biochemical analysis. The study of the ABO and Lewis systems has been greatly facilitated by the discovery that the antigens in question occur not only as surface components of cells, but also are found in water-soluble form in the tissue fluids and secretions of most people.

#### **The ABO and Lewis Systems**

At the turn of the century, Landsteiner first described the existence of serologic differences between individuals<sup>38</sup> allowing him to classify people into one of four groups, depending on whether their red cells contained "agglutinin" A, "agglutinin" B, neither A nor B (O), or both A and B (AB). This

Table 11-1. The ABO Blood Groups

Phenotype (Group)	Genotype	Antigens on Red Cells	Antibodies in Serum
O	OO	None	Anti A Anti B
A	{ AA AO	A	Anti-B
B	{ BB BO	B	Anti-A
AB	AB	A and B	None

discovery led to a series of serologic, genetic, and immunochemical studies that are continuing even at the present time.

**ABO ANTIGENS.**<sup>52,56</sup> The ABO antigens are inherited as simple mendelian characters, the blood group of an individual depending on the presence of two out of three allelic genes: A, B, and O. The possible genotypic and corresponding phenotypic combinations are listed in Table 11-1. The antibodies reacting with the A or B antigen are regularly found in the serum when the corresponding antigen is absent from the red cells. These antibodies were originally thought to occur spontaneously but are now known to arise early in life as a result of exposure to ABO-like polysaccharides that occur ubiquitously in microorganisms, food, and other exogenous sources.

In addition to antibodies against A and B antigens, reagents reacting preferentially with O cells are known. The antigen defined by these reagents is known as H substance and its synthesis is under the control of an allelic pair of genes, H-b, inherited independently of the ABO system. Indeed, since there is no "true" O antigen, group O individuals would be more precisely identified as group H individuals; however, the group O designation is retained for historical reasons.

The H gene in single or double dose gives rise to the H character, and the rare h allele, when present in double dose, results in the absence of the H character. The H-active material appears to be a precursor substance which, under the influence of A and B genes,

is converted into A and B active substances, respectively, thereby accounting for the presence of large quantities of H substance in O individuals. It also follows that the rare hh individuals, termed the "Bombay phenotype,"<sup>56</sup> lack A, B, or H antigens on their erythrocytes and in secretions (Table 11-2). Nevertheless they appear to have normal A and B genes that can be expressed in the next generation if their children acquire an H gene from the other parent.<sup>4,41</sup>

Approximately 80% of northern Europeans with A, B, or H antigens on their red cells also have the corresponding antigen in various tissue fluids and secretions. While the A, B, and H antigens on red cells are predominantly glycolipids (see below), their soluble counterparts in secretions are glycoproteins. The capacity to secrete these substances is under the control of a pair of allelic genes, Se-se. Se is considered dominant over se, and only homozygous sese individuals are therefore "nonsecretors" of A, B, or H substances. Among secretors, the concentration of A, B, and H substances in secretions is influenced by the ABO type of the individual.<sup>79</sup>

**THE LEWIS SYSTEM.** Systematic work on the Lewis system stemmed from the discovery of anti-Le<sup>a</sup> by Mourant in 1946 and anti-Le<sup>b</sup> by Andresen in 1948.<sup>58</sup> Landsteiner and Levine,<sup>39</sup> however, probably first described an example of anti-Le<sup>a</sup>. The Lewis system is closely related to the ABO system, although the two allelic genes, Le and le, which define this system, are inherited independently of the ABO, Hh, and Sese genes.<sup>50</sup> In single or double dose, Le gives rise to the Le<sup>a</sup> specific structure; le in double dose results in its absence. Le<sup>b</sup> specificity, once thought to arise from the activity of an allele of the Le gene, is now considered to be an interaction product between the H and Le genes. Thus, three Lewis red cell phenotypes are possible; these are designated Le(a+b-) or Le<sup>a</sup>, Le(a-b-), and Le(a-b+) or Le<sup>b</sup>.

The Le<sup>a</sup> and Le<sup>b</sup> antigens, unlike their ABH counterparts, are not integral parts of the red cell membrane, but, instead, are acquired from the plasma.<sup>52</sup> Since the secretion

of Le<sup>a</sup> substance is *not* under the control of the Sese gene;<sup>68</sup> nonsecretors have Le<sup>a</sup> antigens on their red cells and in their secretions as long as the Le gene is present. However, since the secretion of H gene products is under the control of the Sese genes, the Le<sup>b</sup> antigen, which is an interaction product between the H gene and the Le gene, is only found in the secretions and on the red cells of secretors (Table 11-2). Thus, the H, Le, and Se genes are *all* required to produce the Le(a-b+) phenotype.

Lewis-negative erythrocytes are readily converted to Le(a+b-) or Le(a-b+) erythrocytes by incubation with the appropriate plasma *in vitro*.<sup>53</sup> Most of the plasma and erythrocyte Lewis antigens appear to be glycosphingolipids and not glycoproteins.<sup>27,53</sup>

Some serious transfusion reactions<sup>56</sup> and a few instances of hemolytic disease due to anti-Lewis antibodies<sup>49</sup> have been reported.

A postulated pathway for the biosynthesis of ABH and Lewis antigens is shown in Figure 11-1.

### Immunochemistry

The chemical composition of *secreted* ABH and of Lewis substances is remarkably similar. Both are composed of a carbohydrate moiety that constitutes about 85% of the molecule and an amino acid moiety that

makes up the remaining 15%.<sup>32,66,66</sup> They are heterodisperse, with an average molecular weight of 300,000,<sup>35,65,66</sup> but the molecules of different sizes have similar composition and molecular properties. The peptide residues are always composed of the same 15 amino acids, and four of these—threonine, serine, proline, and alanine—make up two thirds of all the amino acids present.<sup>12,66</sup> The peptides appear to have structural functions *only* and are thought to form a firm, spiny backbone to which a large number of relatively short oligosaccharide chains, constituting the blood group substances, are attached at intervals.<sup>33,52,66</sup> The carbohydrate moiety of all the ABH and Lewis substances is qualitatively similar in composition.<sup>42</sup> Each contains a methyl-pentose, L-fucose; a hexose, D-galactose; two amino sugars, N-acetyl-D-glucosamine and N-acetyl-D-galactosamine; and a 9-carbon sugar, N-acetyl neuraminic acid. One of these sugars appears to be immunodominant in each of the blood group substances studied.

Cellular blood group substances are much more difficult to isolate, but both A and B active materials have been obtained in water-soluble form. In contrast to the blood group substances found in secretions, most of the cell-derived extracts are glycolipids,<sup>27,83</sup> although some glycoprotein residues have been found as well.<sup>30</sup> Nevertheless, it

Table 11-2. Relation between Genotype, Erythrocyte Phenotype, and Antigenic Specificities Detectable in Secretions

Probable Gene Combination	Antigens Detectable on Red Cells			Specificities Detectable in Secretions		
	ABH	Le <sup>a</sup>	Le <sup>b</sup>	ABH	Le <sup>a</sup>	Le <sup>b</sup>
1 ABO, H, Se, Le	+++	—	++	+++	+	++
2 ABO, H, sese, Le	+++	+++	—	—	+++	—
3 ABO, H, Se, lele	+++	—	—	+++	—	—
4 ABO, H, sese, lele	+++	—	—	—	—	—
5* ABO, hh, Le (se or sese)	—	+++	—	—	+++	—
6* ABO, hh, lele (Se or sese)	—	—	—	—	—	—

\*Groups 5 and 6 correspond to very rare individuals of 'Bombay' phenotype<sup>6</sup> who lack ABH antigens on their red cells and secretions

From Marcus,<sup>52</sup> courtesy of the author and New England Journal of Medicine

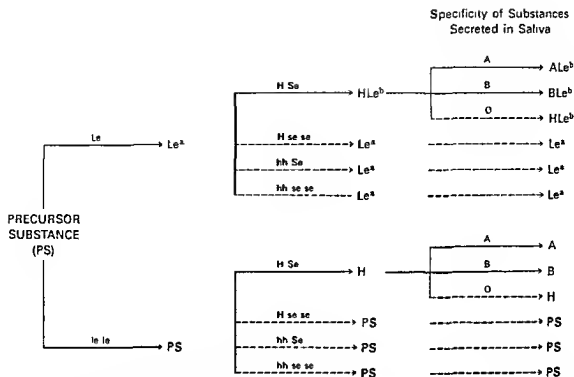


Fig. 11-1 Hypothetical pathway for the biosynthesis of the ABH and Lewis antigenic determinants in glycoproteins under the control of the Le, H, Se and ABO genes. A solid arrow indicates that a sugar residue was added to the glycoprotein, and a broken arrow that no sugar was added. (From Marcus,<sup>52</sup> courtesy of the author and New England Journal of Medicine.)

appears that the oligosaccharide chains that confer blood group specificity on the glycolipid blood group substances of red cells, as well as the glycoprotein substances found in secretions, are identical,<sup>27, 54, 88</sup> thereby accounting for the similarities in the specificity of antigenic determinants on red cells and in secretions.

The complicated interrelationship between A, B, H, and Lewis substances was clarified considerably when it was recognized that the gene systems controlling these four expressions appear to act sequentially on a common glycoprotein precursor substance.<sup>45, 86</sup> Genetic control presumably comes about through the formation of specific glycosyl transferase enzymes or through some mechanism controlling their function. A simplified version of such a proposed scheme is illustrated in Figure 11-2.

Two precursor chains are recognized on the basis of a  $1 \rightarrow 3$  (type 1) or  $1 \rightarrow 4$  (type

2) linkage of the terminal galactosyl unit to the subterminal N-acetyl glucosamine residue.<sup>86</sup> The latter precursor chain has cross-reactivity with antisera to type XIV pneumococcal polysaccharide.<sup>3</sup>

The transferase controlled by the H gene is thought to add L-fucose in  $\alpha$ -linkage to the carbon 2 position of either chain to form an H-active structure.<sup>43, 44</sup> The enzyme controlled by the Le gene, also a fucosyl transferase, adds L-fucose in  $\alpha$ -linkage to the 4 position of the subterminal N-acetylglucosamine unit in chains of type 1 only, as chains of type 2 already are substituted at this position. The resultant structure has  $Le^a$  specificity.<sup>45, 70</sup> Nevertheless, a type 2 oligosaccharide containing fucose linked to C-3 of N-acetylglucosamine may also have very weak  $Le^a$  activity.<sup>45</sup> When both H and Le genes are present, two fucosyl units are added to adjacent sugars on chains of type 1, resulting in a structure which, on the basis of in-

hibition experiments, is thought to be that of the  $\text{Le}^b$  determinant.<sup>55</sup> Type 2 difucosyl chains have weak  $\text{Le}^b$  activity.<sup>55</sup>

The presence of the fucosyl unit conferring H specificity to the precursor substance is considered to be essential for the functioning of the transferases controlled by the A and B genes. Thus, N-acetyl-D-galactosamine added in  $\alpha$ -linkage to carbon 3 of the terminal galactosyl unit confers A-specificity to the H-active precursor substance,<sup>45,70</sup> whereas addition of D-galactose in identical linkage results in B specificity.<sup>43,41</sup> The addition of these terminal nonreducing sugars effectively masks the serologic reactivity of the H-active groupings, and the  $\text{Le}^a$ -active determinants are similarly masked by the substitutions controlled by the H gene. The  $\text{Le}^b$  substance, however, is usually found in good concentration on the red cells and in the secretions of A, B, or AB subjects.<sup>68</sup>

Among individuals who possess the A antigen, two main subclasses have been identified. Subgroup  $A_1$  includes about 80% of Europeans,<sup>56</sup> whereas the remainder belong to subgroup  $A_2$ . Similarly, 80% of AB samples are  $A_1B$ , whereas 20% are  $A_2B$ .  $A_1$  and  $A_1B$  individuals do not form anti- $A_2$  antibodies, but a small proportion of  $A_2$  individuals (1%) and a larger proportion of  $A_2B$  individuals (25%) form anti- $A_1$  antibodies.

The basis for the difference between  $A_1$  and  $A_2$  specificity has been in dispute from the moment of its discovery.<sup>57</sup> One view holds that the  $A_1$  and  $A_2$  antigenic determinants are chemically identical, but that  $A_1$  individuals simply have many more determinants on their red cells than do  $A_2$  individuals.<sup>5,51,57</sup> The other view maintains that there is a qualitative difference between the antigenic determinants of  $A_1$  and  $A_2$  individuals,<sup>27,57</sup> and it has been suggested, on the

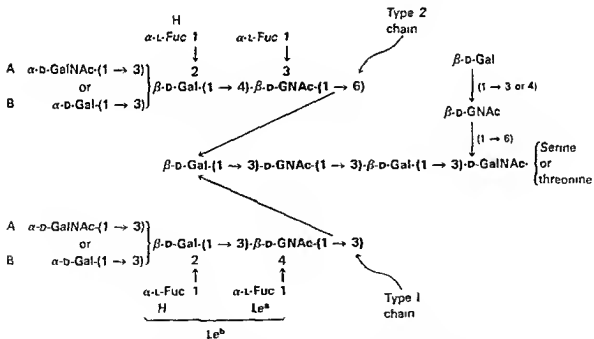


Fig. 11-2 Proposed composite structure of A, B, H,  $\text{Le}^a$  and  $\text{Le}^b$  specific blood group substances linked to serine or threonine spines of the polypeptide backbone. Gal refers to galactose, Fuc to fucose, GNAc to N-acetylglucosamine, GalNAc to N-acetylgalactosamine. The numerical notations (eg 1  $\rightarrow$  4) refer to the respective carbon positions at which linkage occurs. The precursor substance is identified by bold type. The determinants characteristic of a given blood group substance are in light type and are identified by their appropriate symbols, namely A, B, H,  $\text{Le}^a$ , and  $\text{Le}^b$ . The structure shown is a composite which includes all determinants. In individual blood group substances certain residues will be missing: eg H,  $\text{Le}^a$ , and  $\text{Le}^b$  specific blood group substances lack A and B specific determinants, and  $\text{Le}^a$  substance also lacks the H specific determinant. See text and references for details. (From Lloyd et al.,<sup>45</sup> courtesy of the authors and Biochemistry)

basis of immunochemical studies, that  $A_2$  substances lack Type 1 A-determinants<sup>57</sup> (see Fig. 11-2), whereas  $A_1$  substances contain both Type 1 and Type 2 A-determinants.<sup>57</sup> This would also account for the observed higher H and  $Le^b$  activity in  $A_2$  substances, since the addition of the terminal sugar conferring A reactivity ordinarily masks the H or  $Le^b$  active determinants on the precursor chain. Definite proof of molecular differences between  $A_1$  and  $A_2$  determinants will have to await the isolation and structural analysis of  $A_2$  substances.

Numerous variants of  $A_2$  that can be characterized by a variety of serologic techniques have been described.  $A_{31}$ , for instance, is thought to be a "weaker" form of  $A_2$ .<sup>56,71</sup> and even "weaker" forms ( $A_1$ ,  $A_{31}$ ,  $A_m$ ) have been reported.<sup>56-58</sup> These variants are very rare, but may on occasion cause difficulty during typing or cross-matching procedures. The structural basis for these lesser variants, if indeed this is the cause of their poor performance as A antigens, is completely unknown.

While variants of A are encountered with some frequency, variants of B are most uncommon.<sup>18,56,68,89</sup> Occasionally weak B-like antigens have been acquired in association with various diseases, especially malignant ones.<sup>11,23</sup>

**SEROLOGIC SPECIFICITY C.** The classic description of the ABO system and its corresponding isoantibodies (Table 11-1) does not explain a number of puzzling serologic observations.<sup>92</sup> (1) When group O serum is absorbed with A cells, the anti-B titer is lowered as well, and when group O serum is absorbed with B cells, the anti-A titer is decreased also. This phenomenon is not observed when simple mixtures of anti-A and anti-B sera are similarly absorbed. (2) Injection of A cells into O individuals may result in an increased titer of anti-B in addition to the expected rise in anti-A, and vice versa. (3) Eluates of group O serum from group A cells may also agglutinate group B cells, and eluates from B cells may agglutinate A cells.

According to Wiener and associates,<sup>76,92</sup>

group O serum contains, in addition to anti-A and anti-B, antibodies of a third type that react with the blood factor C. In nonimmunized group O subjects the titer of anti-C is generally quite low, but rises sharply following immunization with A or B cells.<sup>92</sup> C factor is present on all red cells having A or B specificity and is also found on the red cells of rare individuals who react with anti-C, though lacking both A and B. When sera containing potent anti-C are absorbed with such "group C" cells, the anti-C activity is removed while anti-A and anti-B activities remain.<sup>92</sup> Absorption with A or B cells, on the other hand, removes the anti-C activity as well.<sup>92</sup> The biochemical structure of the C specificity is unknown.

According to others, Wiener's anti-C, which they term anti-AB, simply reacts with cross-reacting structures common to A and B.<sup>56</sup> These workers have been unwilling to assign a separate specificity to these antisera. Wiener and coworkers consider anti-C to be important in ABO hemolytic disease of the newborn<sup>76</sup> whereas others do not.<sup>94</sup>

### *Distribution of ABH and Lewis Substances*

ABH substances have been found on cells other than erythrocytes. They are found in membranes of all vascular endothelial cells<sup>84</sup> and in many stratified and pseudostratified epithelia, including those of the skin, tongue, esophagus, uterine cervix, and lower genitourinary tract.<sup>84</sup> In addition, blood group substances have been found in the collecting tubules and calyces of secretors,<sup>80</sup> but it is not clear whether they are synthesized by the kidney or merely excreted. When human cell lines possessing ABH antigens are grown in tissue culture, the A and B antigens usually are lost, but the H specificity is retained.<sup>20</sup>

The presence of ABH and Lewis substances in secretions has been mentioned previously. The secretor (Se) gene controls the content of ABH substances in most secretions, including those of the salivary glands, the upper respiratory tract, and the superficial glands of the entire intestinal tract, including

those of the stomach and small intestine. In addition, individuals with the Se gene also have ABH substances in the secretions of the uterine cervix, the prostate, and the lactating mammary gland.<sup>80,84</sup> The mammary gland is of special interest since abundant H substance, but much less A substance and virtually no B substance, is produced by secretors of all ABH phenotypes.<sup>17</sup>

Glands situated more deeply in the mucosa of the pylorus and small intestine, such as Brunner's glands, produce A and B substances in secretors and nonsecretors alike.<sup>25,80</sup> The same is true for gastric parietal glands,<sup>81</sup> the exocrine acini of the pancreas, and the secretory coils of the sweat glands.<sup>89</sup>

Blood group A and B glycoproteins have been isolated from the urine,<sup>34,47</sup> but it is not clear whether they are secreted or simply derived from membrane associated antigens.

### ***Ontogeny of ABH Blood Group Substances***<sup>52,83,85</sup>

ABH substances have been detected as early as the fifth week of embryonal life, when they are found on all vascular endothelia and all epithelia except those of the nervous system, the adrenal gland, and the liver. As differentiation occurs, the antigens progressively disappear, and achieve an adult distribution at the end of the third intrauterine month. Soluble ABH and Lewis substances first appear in the secretions of the salivary glands and the stomach at the end of the second intrauterine month.

### ***ABO Blood Groups and Human Disease***

*Blood transfusion reactions* are discussed later in this chapter. ABO *hemolytic disease of the newborn* is described in Chapter 27.

ABO antigens are known to act as potent *transplantation antigens* in man. It has been found, for instance, that hyperimmunization with incompatible ABO antigens will result in accelerated skin-graft rejection when the donor is incompatible for the same ABO antigen,<sup>36,69</sup> whereas skin grafts from ABO

compatible individuals are accorded survival times characteristic of first grafts in non-sensitized individuals. It has also been found inadvisable to use donor kidneys containing A or B antigens lacking in the recipient.<sup>24</sup> In one study, 46% of kidney transplants with ABO incompatibility failed to show initial function, as compared to only 9% in ABO compatible pairs.<sup>24</sup>

For several decades the ABO groups have been suspected of contributing to *infertility and fetal loss*, but the reports have been conflicting and often speculative. Cumulative evidence now indicates that ABO incompatibility between mother and fetus results in a small but significant decrease in fertility,<sup>40</sup> which may be due to prezygotic selection, prevention of fertilization, or fetal death.<sup>52</sup>

Prevention of fertilization would presumably occur through damage to ABO incompatible sperm by isoantibodies of the cervical secretions.<sup>5,22</sup> About two thirds of all women have these kinds of isoantibodies in their cervical secretions, which are usually IgG and may be produced locally.<sup>64,75,77</sup> The ABO antigens of sperm appear to be acquired from the seminal secretions and are only found on the sperm of secretors.<sup>9,19</sup>

The increased incidence of fetal deaths is usually attributed to fetal damage by maternal IgG antibodies that cross the placenta.

*Alterations in red cell phenotypes* have been observed in a variety of malignant conditions. Loss or suppression of the A<sub>1</sub> antigen has been noted in leukemia.<sup>28,68,71,78</sup> The change is usually confined to red cells and is accompanied by increased H-activity. The loss of the B antigen of an A<sub>1</sub>B patient also has been reported.<sup>72</sup> The acquisition of B-like antigens by patients with malignant disease or infections<sup>23,68</sup> has been mentioned already.

Supposedly significant departures from expected ABO frequencies have been observed in patients with various kinds of disorders. Thus, group A individuals have been reported to be more susceptible to gallstones,<sup>35</sup> cirrhosis of the liver,<sup>7</sup> and tumors of the salivary glands,<sup>61</sup> stomach,<sup>29</sup> and pancreas,<sup>1</sup> as well as ovary<sup>63</sup> than are members of the other groups. In addition, duodenal ulcera-

tion was found to be more common in non-secretors than in secretors of blood group antigens and especially in group O individuals.<sup>13</sup> An increased incidence of myocardial infarction<sup>2</sup> and diabetes mellitus<sup>50</sup> has also been reported in group A individuals. The significance of these studies has been questioned by Wiener and others.<sup>91</sup>

### The Ii System

The red cells of almost all healthy adults carry an antigen I<sup>113,115</sup> and smaller amounts of a related antigen, i.<sup>109</sup> Very rare individuals, perhaps one in 10,000, have little or no I antigen, but they usually have anti-I in their serum.<sup>109</sup> All newborns appear to have the phenotype i since they react strongly with anti-i and weakly with anti-I.<sup>109</sup> It is possible, however, that cord cells may contain I-antigen in a cryptic form since it is possible to isolate I-positive material from such cells.<sup>112</sup> During the first 18 months of life the cells gradually change to the I phenotype and in healthy individuals this reaction pattern appears to be retained throughout life.<sup>109</sup>

Water-soluble I blood group substance has been demonstrated in human saliva, milk, amniotic fluid, and urine.<sup>97,100,110</sup> It has also been found in the saliva of young infants and in the saliva and milk of i-adults who lack the I antigen on their red cells.<sup>99,110</sup> These individuals may nevertheless have potent anti-I in their sera.<sup>110</sup>

In patients with a variety of hematologic disorders, i activity of the red cells may increase again, usually without a demonstrable decrease in I activity. This is particularly true in situations of marrow stress,<sup>105</sup> such as occur in thalassemia major<sup>104</sup> or chronic hemolytic anemia, but it may also be found in hypoplastic anemia<sup>101</sup> and leukemia.<sup>104,108</sup> In the last instance, the i-reactivity of red cells may disappear when the patient achieves a remission.

A relationship between the ABO and Ii systems has been suspected for many years because the red cells of some Oi individuals were found to react very weakly with anti-H sera<sup>113</sup> and because cells of the Bombay phenotype (page 452) usually give a higher

score with anti-I than do ordinary Oi cells.<sup>56</sup> Immunochemical studies with various blood group substances have now confirmed the earlier suspicion.<sup>99,101,102</sup> A complex family of I antigenic determinants has been found in presumed precursors of A, B, H, Le<sup>a</sup>, and Le<sup>b</sup> substances derived from milk, ovarian cyst fluid, and bovine blood group substances. In addition, the careful stepwise degradation of A and B substances yields precursor-like materials with increased I activity. It has therefore been suggested that I specificity appears at intermediate stages in the biosynthesis of A, B, H, Le<sup>a</sup>, and Le<sup>b</sup> substances and that the enzyme produced by the I gene must act on a common precursor just prior to the steps controlled by the A, B, H, and Le<sup>a</sup> genes.<sup>102</sup>

Anti-I antibodies were first described as potent cold agglutinins in the serum of a patient with acquired hemolytic anemia.<sup>115</sup> Anti-I is also found as a weak cold agglutinin in the serum of normal individuals,<sup>56,113</sup> in the serum of most I-negative adults,<sup>113</sup> in patients with certain infections, especially mycoplasma pneumonia, and in patients with idiopathic cold agglutinin disease (Chapter 27). Anti-i is found in patients with malignant disease (Chapter 53) and in association with some infections, especially infectious mononucleosis (Chapter 43), but Wiener could not confirm this last finding.<sup>114</sup>

Anti-I and anti-i sera from various sources have been shown to be distinctly different in specificity as revealed by their reaction patterns against a variety of blood group substances and their precursors,<sup>99,101</sup> pointing to the extraordinary complexity of the I system. One anti-I serum was shown to have maximal specificity for the terminal nonreducing  $\beta$ -D-Gal-(1  $\rightarrow$  4)- $\beta$ -D-GNAc-(1  $\rightarrow$  6) structure present in the type 2 precursor substance (Fig. 11-2). The determinants involved in other I specificities have not been established.<sup>99</sup>

### The Rh System

In 1939 Levine and Stetson published the report of a fascinating observation:<sup>124</sup> the mother of a stillborn baby had a severe



hemolytic reaction to her husband's blood and her serum agglutinated not only her husband's red cells, but also those of 80 out of 104 ABO compatible individuals. The authors correctly suggested that the mother had become sensitized to a "new" antigen, which she lacked herself, but which her stillborn baby had inherited from the father. One year later, Landsteiner and Wiener reported their surprising discovery that the sera of rabbits and guinea pigs immunized with the red cells of Rhesus monkeys agglutinated not only the monkey erythrocytes, but also those of most Caucasian New Yorkers.<sup>123</sup> Individuals whose red cells were agglutinated by these sera were termed "Rh-positive," the others "Rh-negative." These observations suggested the presence of a new system of red cell antigens and stimulated immediate inquiry into the nature of previously unexplained intragroup transfusion reactions and the incompatibilities between mother and child that cause erythroblastosis fetalis (Chapter 27). It was soon demonstrated that most of these reactions could be accounted for by antibodies resembling those described by Landsteiner and Wiener.<sup>136</sup>

From this modest but auspicious beginning has evolved the most complex of human blood group systems, currently characterized by over 30 antigens and antibodies and an almost unlimited number of apparently complex alleles.

Two systems of nomenclature are still in common use and therefore both need to be considered. According to Fisher<sup>127</sup> the inheritance of Rh antigens is determined by three pairs of closely linked allelic genes: Cc, Dd, Ee. Each parent would contribute three genes—C or c, D or d, E or e—each defining a single antigen. Since every person carries a chromosome from each parent, various combinations of genotypes would be found. A person might therefore inherit CDe from one parent and cde from another (CDe/cde), or he may be CDe/CDc, cde/cde, cDe/cde, or CDe/cDe, to mention only some of the most common types. However, so far the postulated anti-d has not been found.<sup>68</sup>

Fisher's theory also assumes that crossover of linked genes can take place, thereby ac-

counting for the maintenance of the rarer combinations by occasional crossing over from common heterozygotes, but there is no convincing evidence to support this suggestion. Moreover, intermediate forms and other variants that do not fit into Fisher's scheme are being discovered; these will be discussed later (page 460).

Wiener's system of nomenclature, which antedates that of Fisher, is based on a different genetic theory, according to which the inheritance of Rh antigens is determined by a series of allelic genes at one locus.<sup>137,138</sup> This means that the inheritance of Rh antigens is determined by a single gene, rather than three separate ones, as postulated by Fisher. This theory now is considered to be correct.<sup>36</sup>

In order to understand Wiener's nomenclature, two terms need to be explained. The term "agglutnogen" is used to describe the whole antigen complex determined by a given gene. Unfortunately its structure and physicochemical properties are not yet known, but it can nevertheless be identified by its "blood factors," the serologic specificities defined by antibodies directed against various facets of its structure. According to Wiener, the terms "blood factor" and "antigenic determinant" are not synonymous, since one and the same antigenic determinants may be identified by different families of antibodies in slightly different ways,<sup>138,139</sup> just as one blind person might identify a face by its nose, while another identifies the same face by its nose and its mouth, and yet another by its mouth and its ears. In his own publications, Wiener uses italics for gene symbols and genotypes, regular type for agglutinogens and phenotypes, and boldface type for blood factors and their corresponding antibodies (not shown in Table 11-4). In addition, the letter h is omitted from gene symbols and only superscripts are used.

An example will help to illustrate the difference between the Wiener and Fisher-Race terminologies. According to Wiener, a single gene R<sup>2</sup> determines the "agglutnogen" Rh<sub>2</sub> (cDE), which may be identified by its corresponding "blood factors" Rh<sub>0</sub> (D), rh"

(E), and hr' (c). According to the Fisher-Race system, the phenotype cDE is the result of three closely linked genes, c, D, and E, that an individual has inherited from one of his parents. One of the drawbacks of the Wiener terminology is its apparent complexity. In addition, one cannot deduce the nature of constituent blood factors from the notation for a given agglutinin. The factors have to be memorized. On the other hand, the Fisher-Race terminology is deceptively simple, implying, for instance, that cDE contains only c, D, and E, whereas it may in fact contain other "blood factors" as well.

The most important blood factors of Wiener and their corresponding Fisher-Race notations as well as the approximate frequencies of the various blood factors in Caucasians are given in Table 11-3. Wiener's gene designations, corresponding agglutinogens, and blood factors, as well as the corresponding Fisher-Race notations are given in Table 11-4.

The observed blood type of each individual is the product of a pair of genes. When the eight genes listed in Table 11-4 (Wiener's notation) are paired with each other, 36 genotypes are possible. The number of observed phenotypes is considerably less, however, since in each combination a factor may be represented once or twice, or two different

factors may be on the same or on different agglutinogens, without changing the phenotype. Thus, using the six antisera listed in Table 11-5, 20 Rh-Hr "phenotypes" can presently be identified.

In addition to the 6 Rh-Hr factors listed in Table 11-5, an ever expanding number of rare variants continues to add complexity to the Rh-Hr system. These include: (1) alternatives to common factors (allelic antigens); (2) compound antigens or, according to the Fisher-Race interpretation, joint products of theoretically separable genes; (3) deletions or suppression of some Rh antigens; (4) Rh null; (5) the LW antigen.

**ALLELIC ANTIGENS.** These variants appear to function as cognates for one of the more common factors. Thus  $rh^{W1}(C^W)$  and  $rh^X(C^X)$  appear to be associated with  $rh'(C)$ ,  $rh^{W2}(E^W)$  appears to be associated with  $rh''(E)$ , and  $D^U$  and  $D^W$  substitute for D. In addition,  $Rh_0(D)$  and  $rh''(E)$  cognates with missing components have been described.

$rh^{W1}(C^W)$  occurs in 2% of Caucasians, but in certain northern European population groups, such as Latvians, Finns, and Lapps, incidence rates as high as 7 to 9% are found.<sup>68</sup>  $rh'(C)$  individuals may make antibodies against the  $rh^{W1}(C^W)$  factor. In contrast, the  $rh^X(C^X)$  variant is rare (0.03% in Caucasians). Apparently the corresponding antibody may occur in individuals suffering from acquired hemolytic anemia.<sup>68</sup>

$Rh_0(D^U)$  appears to be the product of variant  $R^0$  genes, which result in a series of rather "weak"  $Rh_0(D)$  antigens. Alternatively it has been suggested that some of these "weak" antigens may be due to a position effect exerted by  $rh'$  on the ordinary  $Rh_0$  in the opposite gene complex, as in the genotype  $R^0r'$ .<sup>119</sup> Gradations of reactivity with anti  $Rh_0(D)$  sera have been reported; while the cells of some  $Rh_0(D^U)$  individuals are agglutinated by certain antisera only, the cells of others are not, and are serologically indistinguishable from  $r'r$  ( $Cdc/cdc$ ) or  $r'r$  ( $cDE/cde$ ) cells,<sup>68</sup> except by a positive indirect anti-globulin response with incomplete anti-D sera.<sup>68</sup> The antigen is of practical importance

Table 11-3. Wiener<sup>138</sup> and Fisher-Race<sup>68</sup> Rh Terminology

Rh Hr Blood Factors	CDE Terms	Approximate Frequencies of Blood Factors in Caucasians (%)
$Rh_0$	D	85
$rh'$	C	70
$rh''$	E	30
$hr'$	c	80
$hr''$	e	97
$hr$	f, ce	64
$rh_1$	Ce	69
$rh^{W1}$	$C^W$	2
$rh^X$	$C^X$	0.03
$hr^V$	V, ce <sup>a</sup>	<1
$rh^{W2}$	$E^W$	very rare
$rh^G$	G	87

**Table 11-4. Wiener's Gene Designations, Corresponding Agglutinogens and Blood Factors, and Fisher-Race Notations**

Genes		Corresponding Agglutinogens	Blood Factors Present	Frequency among Caucasian (%)
Fisher Race	Wiener			
<i>cde</i>	<i>r</i>	<i>rh</i>	<i>hr', hr'', hr</i>	38.0
<i>Cde</i>	<i>r'</i>	<i>rh'</i>	<i>rh', hr''</i>	0.6
<i>cdE</i>	<i>r''</i>	<i>rh''</i>	<i>rh'', hr'</i>	0.5
<i>CdE</i>	<i>r<sup>0</sup></i>	<i>rh<sup>0</sup></i>	<i>rh' rh''</i>	0.01
<i>cDe</i>	<i>R<sup>0</sup></i>	<i>Rh<sub>0</sub></i>	<i>Rh<sub>0</sub> hr', hr'', hr</i>	2.7
<i>CDE</i>	<i>R<sup>1</sup></i>	<i>Rh<sub>1</sub></i>	<i>Rh<sub>0</sub> rh' hr''</i>	41.0
<i>cDE</i>	<i>R<sup>2</sup></i>	<i>Rh<sub>2</sub></i>	<i>Rh<sub>0</sub> rh'' hr'</i>	15.0
<i>CDE</i>	<i>R<sup>3</sup></i>	<i>Rh<sub>3</sub></i>	<i>Rh<sub>0</sub> rh', rh''</i>	0.2

Wiener, in his publications uses italics for gene symbols and for genotypes regular type for agglutinogens and phenotypes, and bold faced type for symbols for blood factors and for the corresponding antibodies used to detect the blood factors in question. Further, to distinguish the symbols for genotypes and phenotypes, the letter 'h' is omitted and only superscripts are used in gene symbols.

because  $Rh_0(D^U)$  is common in Negroes, because it can stimulate anti- $Rh_0(D)$  antibodies in type *rh* individuals and because some  $Rh_0(D^U)$  individuals have made anti- $Rh_0(D)$  antibodies.<sup>63</sup>

Variants of  $Rh_0$  with increased reactivity have been reported in rare instances and will

be discussed in a subsequent section (page 462).

**OTHER COGNATES OF  $Rh_0$  FACTORS.** It has been known for some time that a few  $Rh_0(D)$  individuals can make antibodies simulating anti  $Rh_0(D)$  in specificity.<sup>123</sup> It has been

**Table 11-5. The Rh Phenotypes and Their Corresponding Genotypes**

Phenotype	Reaction with						Corresponding Genotype
	Anti- $Rh_0$	Anti <i>rh'</i>	Anti- <i>rh''</i>	Anti- <i>hr'</i>	Anti <i>hr''</i>	Anti- <i>hr</i>	
<i>rh</i>	—	—	—	+	+	+	<i>rr</i>
<i>rh'rh</i>	—	+	—	+	+	+	<i>r'r</i>
<i>rh'rh'</i>	—	+	—	—	+	—	<i>r'r'</i>
<i>rh''rh</i>	—	—	+	+	+	+	<i>r''r</i>
<i>rh''rh''</i>	—	—	+	+	—	—	<i>r''r''</i>
<i>rh'rh''</i>	—	+	+	+	+	—	<i>r'r''</i>
<i>rh<sub>0</sub>rh</i>	—	+	+	+	+	+	<i>r'r</i>
<i>rh<sub>0</sub>rh'</i>	—	+	+	—	+	—	<i>r'r'</i>
<i>rh<sub>0</sub>rh''</i>	—	+	+	+	—	—	<i>r'r''</i>
<i>rh<sub>0</sub>rh<sub>0</sub></i>	—	+	+	—	—	—	<i>r'r'</i>
<i>Rh<sub>0</sub></i>	+	—	—	+	+	+	<i>R<sup>0</sup>R<sup>0</sup></i> and <i>R<sup>0</sup>r</i>
<i>Rh<sub>1</sub>rh</i>	+	+	—	+	+	+	<i>R<sup>1</sup>r, R<sup>1</sup>R<sup>0</sup></i> and <i>R<sup>0</sup>r'</i>
<i>Rh<sub>1</sub>Rh<sub>1</sub></i>	+	+	—	—	+	—	<i>R<sup>1</sup>R<sup>1</sup></i> and <i>R<sup>1</sup>r'</i>
<i>Rh<sub>2</sub>rh</i>	+	—	+	+	+	+	<i>R<sup>2</sup>r, R<sup>2</sup>R<sup>0</sup></i> and <i>R<sup>0</sup>r''</i>
<i>Rh<sub>2</sub>Rh<sub>2</sub></i>	+	—	+	+	—	—	<i>R<sup>2</sup>R<sup>2</sup></i> and <i>R<sup>2</sup>r''</i>
<i>Rh<sub>3</sub>Rh<sub>2</sub></i>	+	+	+	+	+	—	<i>R<sup>1</sup>R<sup>2</sup>, R<sup>1</sup>r''</i> and <i>R<sup>2</sup>r'</i>
<i>Rh<sub>3</sub>rh</i>	+	+	+	+	+	+	<i>R<sup>3</sup>r, R<sup>3</sup>R<sup>0</sup></i> and <i>R<sup>0</sup>r'</i>
<i>Rh<sub>3</sub>Rh<sub>1</sub></i>	+	+	+	—	+	—	<i>R<sup>1</sup>R<sup>3</sup>, R<sup>1</sup>r'</i> and <i>R<sup>3</sup>r'</i>
<i>Rh<sub>3</sub>Rh<sub>2</sub></i>	+	+	+	+	—	—	<i>R<sup>1</sup>R<sup>2</sup>, R<sup>1</sup>r''</i> and <i>R<sup>2</sup>r'</i>
<i>Rh<sub>3</sub>Rh<sub>3</sub></i>	+	+	+	—	—	—	<i>R<sup>3</sup>R<sup>3</sup></i> and <i>R<sup>3</sup>r'</i>

postulated that these individuals lack some part of the normal  $Rh_0$  factor and make antibodies to this missing part. Wiener suggested that the  $Rh_0$  factor may have associated with it a series of factors,  $Rh^A$ ,  $Rh^B$ ,  $Rh^C$ ,  $Rh^D$ . He indicates missing specificities by a small letter, for instance,  $Rh^c$ ,  $Rh^{c'}$ , etc.<sup>135</sup> It has been found that typically reacting  $Rh_0$  samples in blacks and whites seldom lack constituent components; however, about half of the  $Rh_0(D^U)$  bloods tested lack one or more of the cognate factors.<sup>135</sup>

The specificity  $E^T$  appears to be missing from the  $rh''(E)$  antigen of about one third of Australian aborigines living in the Western Desert.<sup>68</sup> An anti- $E^T$  antibody, apparently naturally occurring, was found in the serum of an Australian aborigine of the genotype  $R^1R^2(CDe/CDe)$ .

**COMPOUND ANTIGENS.** These are produced when two standard factors, both situated on the same agglutinin, combine determinants to give rise to a new specificity. The original factors,  $hr'$  (c) and  $hr''$  (e), have to be in the "cis-position" with respect to their agglutinin. (Cis-position simply indicates that the blood factors are products of the same gene and are therefore situated on the same agglutinin. Similarly, factors situated on different agglutinins and derived from opposing chromosomes are said to be in the "trans-position.") The resulting factor  $hr$  (ce) gives rise to antibodies that do not react with  $hr'$  (c) or  $hr''$  (e) separately. This compound factor has previously been referred to as anti-f or anti-Ce.<sup>120,128</sup>

Other "compound" antigens may include  $rh_1(Ce)$ ,  $rh_2(cE)$ ,  $rh_2(CE)$  and, perhaps, several others.<sup>68</sup>

**SUPPRESSIONS.** There are rare but theoretically important agglutinogens with part or all of their factors missing. Thus the genetically determined agglutinin  $Rh_0(D-)$  lacks factors at both the  $rh'-hr'$  (Cc) and  $rh''-hr''$  (Ee) positions and has instead greatly increased  $Rh_0(D)$  activity.<sup>68</sup> The  $Rh_{null}$  phenotype may represent an extreme example of this type of suppression (see below). Other rare examples of suppression have been summarized by Race and Sanger.<sup>68</sup>

$Rh_{null}$ .<sup>122,126,131</sup> This is a most unusual blood type that lacks all Rh-Hr factors. In addition, aberrations of the MNSsU system have been reported.<sup>131</sup> Two types of  $Rh_{null}$  have been postulated. One is thought to result from the effect of a suppressor gene (z), when the person is homozygous for that gene.<sup>126</sup> Thus an  $R'r$  zz individual would react as  $Rh_{null}$  and his blood is designated  $\bar{r}h$ . Alternatively,  $Rh_{null}$  could be the result of homozygosity for an allelic gene  $\bar{r}$ . This would result in the genotype  $\bar{r}\bar{r}$  and the blood would be designated  $\bar{r}h$ .<sup>122</sup> Others consider  $Rh_{null}$  to be the result of gene deletions.<sup>131</sup>

$Rh_{null}$  individuals are of added interest to hematologists, since they suffer from chronic hemolytic anemia characterized by spherocytosis, stomatocytosis, "smiling erythrocytes," and increased osmotic fragility.<sup>132</sup> No intracorpuscular or other membrane defects have so far been demonstrated.

The anti-Rh antibodies are described in Chapter 27.

**THE LW ANTIGEN.** In 1940, Landsteiner and Wiener discovered that the sera of rabbits and guinea pigs immunized with the red cells of Rhesus monkeys also agglutinated the erythrocytes of most Caucasian New Yorkers (see above). According to Wiener this antibody reacts with antigens presently known to be part of the Rh system,<sup>140</sup> but others claim that it reacts with a different antigen, LW (named in honor of Landsteiner and Wiener<sup>125</sup>), which is shared by human, baboon, and Rhesus cells.<sup>56,68</sup> Alleged differences between anti-LW and anti-Rh sera have been summarized by Mollison<sup>56</sup> and by Race and Sanger.<sup>68</sup> Wiener has denounced all these claims in the strongest terms.<sup>140</sup> He maintains that Landsteiner and Wiener's heterologous anti-Rh sera prepared in rabbits and guinea pigs simply detect another blood factor on the Rh agglutinin, thereby demonstrating again the multiple serologic specificities of a single agglutinin molecule.<sup>141</sup>

## MN, Ss, U System

The M and N antigens were discovered by Landsteiner and Levine in 1927.<sup>193</sup> Rabbits

were immunized with human red cells and, in addition to species specific antibodies, these animals developed antibodies that agglutinated some human cells (M) but not others (N). When the inagglutinable cells were used to immunize rabbits, antisera that specifically agglutinated these (N) cells resulted. Appropriately absorbed antisera are capable of identifying three red cell phenotypes: M, N, and MN, corresponding to the MM, NN, and MN genotypes respectively (Table 11-6). Human M and N antigens are thus distinguished by specific antibodies evoked in rabbits. They are poorly immunogenic in man; naturally occurring anti-M is rare and anti-N even rarer.<sup>56</sup> Occasionally anti-M is found as an immune antibody, usually after multiple transfusions or deliberate sensitization with M-positive blood.<sup>56</sup> Under such circumstances anti-M (and anti-N) antibodies are usually IgM in nature and have a thermal amplitude of 4 to 20° C, seldom extending to 37° C. They may therefore be detected as cold agglutinins. IgG anti-M antibodies have, however, been described.<sup>56</sup>

Very rarely hemolytic disease of the newborn due to anti-M may be noted, but since the infant would of necessity have to be type MN, the disease is either very mild or does not occur at all, as MN cells react more weakly with anti-M than do M cells.<sup>56</sup> In addition the antibody would have to be IgG in nature.

It has been suggested that the synthesis of M-N antigens is, in a way, similar to that of the A-B-H system, being the result of sequential gene-controlled changes in a common precursor substance.<sup>193,207</sup> Studies sup-

porting this view<sup>193,207</sup> were carried out with specific anti-M and anti-N sera of animal and human origin and with extracts from *Vicia graminea*, a leguminous plant, which interact with N-specific structures. It appears that both M- and N-activities are associated with the same macromolecule and that N-activity is found in M-preparations derived from cells with the genotype MM. Furthermore, mild acid hydrolysis of M-substance leads to a loss of M-specificity and uncovering of N-specificity. The decrease in M-activity and increase in N-activity are accompanied by a loss of N-acetyl neuraminic acid (NANA), suggesting that terminal NANA groups play a role in M-specificity. Treatment with neuraminidase, which removes all NANA from the red cell surface destroys not only M- but also N-activity. However, in contrast to the N-activity of M-antigen, N-antigen possesses no M-activity. Treatment of N-specific structures with  $\beta$ -galactosidase destroys all N-activity with the release of galactose, and some human anti-N sera are inhibited by galactose and other sugars possessing  $\beta$ -D-galactopyranosyl groupings, suggesting that  $\beta$ -D-galactopyranosyl structures, in addition to NANA, contribute significantly to N-specificity. This is further supported by the inhibitory effect of asialoganglioside and ganglioside I on anti-N sera.<sup>207</sup> Since treatment with neuraminidase destroys all M- and N-activity, NANA must also play a role in N-specificity.

The *Vicia* reagent, which is known to interact with N-specific structures, reacts even more strongly with partially hydrolyzed N- (and M-) antigens. The specificity of *Vicia* reagents is not dependent on NANA, but on terminal  $\beta$ -D-galactopyranosyl residues.

Thus, the following pathway for the biosynthesis of M- and N-specificities has been proposed<sup>207</sup>: The product of the N-blood group gene is the immediate precursor of the product of the M-blood group gene, while the allele to the M-gene is an amorph. The structure reacting with the *Vicia* reagent seems to lie in the biosynthetic pathway of the MN-macromolecule and appears before the N- or A1-determinants. The myxovirus receptor properties of these structures<sup>193</sup> ap-

Table 11-6. The MNSs System

Genotype	Phenotype	Frequency
MSMS	MS	5 7
NSNS	NS	0 3
MSNS	MNS	3 9
MsMs	Ms	10 1
NsNs	Ns	15 6
MsNs	MNs	22 6
MSMs	MSs	14 0
NSNs	NSs	5 4
MSNs (MsNs)	MNSs	22 4

pear concurrently with the N-group specificity.<sup>207</sup>

In 1947 an antiserum, designated anti-S, was discovered and was shown to react with about 55% of human red cells.<sup>193</sup> An anti-s serum that reacts with about 90% of human red cells was found later. The antigens defined by these sera appear to be the products of allelic genes, and although the Ss antigens cut across the MN distribution, a close association with the MN system can be demonstrated, with a bias towards MS/MS and Ns/Ns.<sup>68</sup>

U, an almost universal antigen present in all Caucasians and most Negroes, was discovered in 1953 as the result of a fatal transfusion reaction in a Negro woman.<sup>220</sup> The serum was designated anti-U. Anti-U is not simply anti-Ss, as was formerly believed, since anti-U sera will react with some cells of the type M<sub>s</sub>S-negative, s-negative, U-positive.<sup>26</sup> The allele u is at least relatively common in Negroes (1%) but is not found in Caucasians. Individuals lacking the U antigen are designated S<sup>u</sup>S<sup>u</sup>. Uu is closely associated with the Ss system since no S or s antigens have so far been detected in u individuals. It has been suggested that U might be a precursor substance for Ss antigens.

Anti-S may be present in patients who have received many transfusions and has resulted in fatal transfusion reactions. Occasionally it is found as a naturally occurring antibody. It may also cause hemolytic disease of the newborn. Some anti-S sera react only with SS red cells.<sup>193</sup>

Anti-s is very rare, but may cause hemolytic disease of the newborn. Anti-U, also rare, may arise following transfusion or pregnancy and has been associated with hemolytic disease of the newborn as well.<sup>56</sup>

**MN VARIANTS.** A large number of MNSs variants have been described. M<sub>1</sub> differs qualitatively from M, and appears to be related to M as A<sub>1</sub> is related to A. It is common in American Negroes (25% of M bloods), but only 5% of Caucasian M bloods are M<sub>1</sub>. The antigen tends to have a weaker intrinsic N content. Anti-M<sub>1</sub> sera are found in N indi-

viduals only and some have dual specificity (anti-M + anti-M<sub>1</sub>).

M<sup>k</sup> is a rare variant that lacks all MNSs, U, and other factors and is therefore analogous to the Rh<sub>null</sub> agglutinin.<sup>181</sup> Very weak antisera can be produced in rabbits and are occasionally found in man. M<sup>k</sup> individuals also suffer from a red cell membrane defect that causes red cells to agglutinate in "incomplete" antisera<sup>210</sup> (Chapter 27).

M<sup>s141</sup> is extremely rare outside Switzerland, where the distribution is 0.153%. The antigen gives no reaction with anti-M or N sera. Anti-M<sup>s</sup> is one of the commonest antibodies of the MNSs system, occurring in 1 to 4% of tested individuals. Because the antigen is so rare, however, it has not caused serious problems in blood matching.

A number of other rare variants have been described including He,<sup>191</sup> Hu,<sup>191</sup> M<sub>2</sub>,<sup>191</sup> M<sup>6</sup>, M<sup>7</sup> and M<sup>8</sup>,<sup>183</sup> M<sup>10</sup>,<sup>176</sup> N<sub>2</sub>,<sup>175</sup> N<sub>3</sub>,<sup>191</sup> R<sup>1</sup>,<sup>155</sup> S<sup>1</sup>,<sup>180</sup> V<sup>1</sup>,<sup>170</sup> and Vw (Gr).<sup>183</sup>

### The P System

In 1927, Landsteiner and Levine described yet another system by injecting human red cells into rabbits.<sup>56</sup> Human red cells reacting with these antisera were called P+, and those not reacting, P-.

The P blood group system is now thought to be determined by three alleles, P<sup>1</sup>, P<sup>2</sup>, and the very rare gene p, which in double dose results in the absence of P<sub>1</sub> and P<sub>2</sub> antigens<sup>150</sup> (Table 11-7). The recognition of increasing complexity within this system was largely due to the discovery of a rare antiserum,

**Table 11-7. Approximate Frequency of P-Phenotypes and Their Antibodies<sup>56,58</sup>**

Phenotype of Cells	Frequency of Phenotype	Antibody in Serum	Frequency of Antibody
P <sub>1</sub>	75%	—	—
P <sub>2</sub>	25%	Anti P <sub>1</sub>	90%*
P <sup>k</sup>	Very rare	Anti-P + P <sub>1</sub>	?
p	Very rare	Anti-P + P <sub>1</sub> + P <sup>k</sup> (Anti Tj <sup>a</sup> )	100%

\*At 0°C

Table 11-8. Reactions of Red Cells with Antibodies of the P System\*

Phenotype of Cells	Antibodies		
	Anti- $P_1$	Anti-P	Anti-P + $P_1$ + $P^k$
$P_1$	+	+	+
$P_2$	-	+	+
$P^k$	- or +	-	+
p	-	-	-

\*Also, see Wiener's interpretation of this system.<sup>217</sup>

anti-Tj<sup>a</sup>, which was soon shown to react with red cells of  $P_1$  and  $P_2$  phenotypes and was later also shown to contain anti- $P^k$  antibodies.<sup>177</sup> Wiener considers the evidence for a separate  $P^k$  antigen weak and has proposed a system of only two isoantibodies, anti-P and anti- $p'$ .<sup>217</sup>

The potential clinical importance of the P blood group system derives from the fact that antibodies may occur in the serum of individuals who lack the corresponding antigen (Tables 11-7 and 11-8). Thus, the anti- $P_1$  sera are found in  $P_2$  donors and react specifically with  $P_1$  cells. Anti-P sera react with  $P_1$  or  $P_2$  cells, but when these sera are absorbed with  $P_2$  cells, only anti- $P_1$  activity remains. Anti-P antibodies are found in pp and  $P^k$  subjects. Anti-Tj<sup>a</sup> reacts with  $P_1$ ,  $P_2$ , and  $P^k$  determinants and is found in individuals of the pp genotype.

Clinically significant transfusion reactions due to anti- $P_1$  must be exceedingly rare, although rapid destruction of isotopically labeled cells has been demonstrated.<sup>56</sup> Anti-P +  $P_1$ , found in pp and  $P^k$  individuals and as the Donath Landsteiner antibody in paroxysmal cold hemoglobinuria (Chapter 27), is very rare; it occurs predominantly in offspring from consanguineous marriages and in isolated geographic areas.<sup>68</sup>

$P_1$  antigen activity is thought to be associated with a glycosphingolipid structure<sup>192</sup> and D-galactose in  $\alpha$ -linkage appears to be the immunodominant sugar.

### Other Systems

**THE KELL SYSTEM.** The most important antigen in this system is K. Anti-Kell anti-

bodies were first found in the serum of a mother (Mrs. Kell) whose child probably had erythroblastosis fetalis,<sup>63</sup> and independently in a patient with a hemolytic transfusion reaction.<sup>218</sup> By means of an antibody recovered from the mother (Mrs. Cellano) of another child suffering from erythroblastosis fetalis,<sup>179</sup> a second antigen that was very common (99.8%) was found; this appeared to be allelic with K. It was therefore designated k. In England, 0.2% of the population have the genotype KK, 8.7% are Kk, and 91.1% are kk.<sup>56</sup> The first two genotypes are phenotypically identified as K-positive, the latter K-negative.

Other antigens of the Kell system include the antigens Kp<sup>a</sup> and Kp<sup>b</sup>,<sup>145,151</sup> and Sutter<sup>163</sup> which is defined by the antigens Js<sup>a</sup> and Js<sup>b</sup>.<sup>186,209</sup> Js<sup>b</sup> and Kp<sup>b</sup>, like k, have a very high frequency, but about 20% of Negroes are Js<sup>a</sup> positive. Kw is also part of the Kell system.<sup>56</sup> It is found in 6% of Caucasians and in 24% of Negroes. In the extremely rare phenotype K<sub>0</sub>, none of the above antigens is present.<sup>151</sup>

Naturally occurring anti-K antibodies are exceedingly rare,<sup>56</sup> but Kell-negative individuals may become sensitized following Kell-positive transfusions. Fortunately the Kell antigen is not as immunogenic as the Rh<sub>0</sub> antigen.<sup>221</sup> Production of anti-K antibodies by pregnant women is a relatively rare event, and most of those who have done so have had a history of previous transfusion, sometimes with blood from their husbands!<sup>56</sup> Once formed, anti-K is a potent cause of hemolytic disease of the newborn and may lead to severe transfusion reactions, even with acute renal failure.<sup>56,166</sup>

Anti-K antibodies may be either IgG or IgM, although they usually fail to agglutinate red cells suspended in saline solution. The antibody is best detected by the indirect Coombs' test.<sup>163</sup> In this system, IgG antibodies are most readily demonstrated by specific anti-IgG sera, whereas the presence of IgM antibodies is best detected by anti-complement sera.<sup>56</sup>

**THE KIDD SYSTEM.** This system, defined

by the two antigens  $Jk^a$  and  $Jk^b$ , was discovered in the study of a mother whose child had hemolytic disease of the newborn.<sup>63</sup> Like Kell, it behaves genetically as a two-allele system, but it also has a silent allele of infrequent occurrence that gives rise to the phenotype  $Jk(a-b-)$ . Approximately half the population has the phenotype  $Jk(a+b+)$ , a quarter are  $Jk(a-b+)$ , and a quarter are  $Jk(a+b-)$ .<sup>194</sup>  $Jk^a$  and  $Jk^b$  are rather weak antigens, but several instances of isoimmunization have been recorded, usually following transfusions.<sup>56,63,198</sup> Anti- $Jk^a$  is the more common antibody. In its presence, severe transfusion reactions may occur.

Anti- $Jk^a$  antibodies bind complement and are best demonstrated by the indirect antiglobulin test, especially if fresh complement is added.<sup>56</sup> The antibodies are usually IgG, but may be IgM.<sup>193</sup>

**THE LUTHERAN SYSTEM.** This is defined by an allelic pair of genes,  $Lu^a$  and  $Lu^b$ . The common phenotypes and their approximate frequencies are  $Lu(a-b+)$ , 92.35%;  $Lu(a+b+)$ , 7.5%; and  $Lu(a+b-)$  0.15%.<sup>63</sup> The frequency of the  $Lu^a$  gene appears to increase as one goes northward in Europe and varies between 2.1 and 9.1%, being highest in Danes and Norwegians.<sup>171</sup> It is found in Negroes, but is very rare in members of other races.<sup>68</sup> A rare silent phenotype,  $Lu(a-b-)$ , also has been encountered.<sup>158</sup>

$Lu^b$  appears to be more antigenic than  $Lu^a$ . Anti- $Lu^b$  has caused mild hemolytic transfusion reactions.<sup>156</sup> It may also have been responsible for a case of hemolytic disease of the newborn.<sup>56</sup> The anti- $Lu^a$  antibody is rare and has not been implicated in hemolytic disease. Most examples of anti- $Lu^a$  appear to represent "naturally occurring" antibodies. The antibody behaves as a complete agglutinin and is presumably IgM.<sup>56</sup>

**THE DUFFY SYSTEM.**<sup>157</sup> This consists of the alleles  $Fy^a$ ,  $Fy^b$ ,  $Fy$ , and possibly  $Fy^x$ .<sup>152</sup> The various genotypes and phenotypes, as well as their relative frequencies, are listed in Table 11-9. Of all the known blood group genes,  $Fy$  is thought to provide the greatest distinc-

Table 11-9. Duffy Genotypes and Phenotypes (in England)<sup>56</sup>

Genotype	Phenotype	Reaction with		Frequency (%)
		Anti- $Fy^a$	Anti- $Fy^b$	
$Fy^a Fy^a$	$Fy(a+b-)$	+	-	17
$Fy^a Fy^b$	$Fy(a+b+)$	+	+	49
$Fy^b Fy^b$	$Fy(a-b+)$	-	+	34
$Fy Fy$	$Fy(a-b-)$	-	-	0.3

tion between Negroes and Caucasians, its frequency being 83% in New York blacks, 90% in West Africans, and 3% in Europeans.<sup>69</sup>

$Fy^a$  appears to be a much "stronger" antigen than  $Fy^b$ ; sensitization is usually due to prior transfusion and has resulted in severe transfusion reactions as well as hemolytic disease of the newborn.<sup>56,68,167</sup> Anti-Duffy antibodies are best detected by the indirect Coombs' test. The antibody is usually IgG and frequently binds complement.<sup>56</sup>

Enzyme treatment of red cells, especially when impure preparations are used, may destroy the Duffy antigens, and enzyme matching and antibody techniques may therefore not detect anti-Duffy antigens or antibodies.<sup>56</sup> This apparently is not true when crystalline trypsin is used.<sup>56</sup>

**CHIDO.** The Chido antigen has a frequency of about 98%<sup>56</sup> and it is found in the plasma<sup>182</sup> as well as on red cells and, possibly, on the surface of leukocytes.<sup>215</sup> In cord blood, Chido antigen is found in the plasma but is very weakly expressed in red cells.<sup>182</sup>

The Chido antibody may cause difficulty in cross-matching in previously transfused patients.<sup>56</sup> The antibody gives a range of reactions and it is sometimes difficult to distinguish Chido-negative donors from those Chido-positive donors whose red cells react weakly or not at all.<sup>182</sup> This difficulty may be resolved by using an inhibition test based on the presence of Chido substance in the plasma of Chido-positive individuals.<sup>182</sup>

**DIEGO.** The antigen  $Di^a$  (Diego) defines another locus controlling antigen of the red



cells and saliva.<sup>178</sup> This antigen is not found, except as an extreme rarity, in the blood of Europeans and West Africans, but it is found in the blood of South American Indians, Japanese, and Chinese, and is generally presumed to be a Mongolian character. Its near absence in Eskimos is puzzling. Like other blood group antigens, it is inherited as a dominant character.

AUBERGER. This is a blood group that is found in 82% of Caucasians and probably in the same proportion of Negroes.<sup>200</sup> This antigen may be associated with the Lutheran system, since some Lu(a-b-) subjects are also Auberger-negative.<sup>56</sup>

"PRIVATE" AND "PUBLIC" ANTIGENS. In addition to the blood group systems described above, a number of very infrequent ("private") antigens and some common ("public") ones have been identified.

"Private" antigens<sup>55, 68</sup> include Levay, Wra, Be<sup>a</sup>, By, Rm, and others. They were discovered when they stimulated the production of an antibody by transfusion or when they caused hemolytic disease of the newborn. These rare antigens sometimes prove to be heralds of a new blood group system, as in the case of Wright (Wra), but sometimes they are rare members of established systems.

"Public" antigens are those that are possessed by the vast majority of people. These include Vel, Yr<sup>a</sup>,<sup>160, 173</sup> Sm,<sup>203</sup> Cs<sup>a</sup>,<sup>164</sup> and others.<sup>68</sup> Vel is of interest because it must be kept in mind when patients are not readily cross-matched.<sup>212</sup> Vel sensitization is known to produce mild to severe transfusion reactions, but hemolytic disease of the newborn has not been reported.

NEW GROUPS. New blood groups continue to be reported: Bu<sup>a</sup>,<sup>146</sup> Do<sup>a214</sup> and Do<sup>b</sup>,<sup>185</sup> Kamhuber,<sup>206</sup> etc. It would be rash to consider that the end of the list is in sight. The most interesting of the more recent crop is Xg<sup>197, 202</sup> for this is carried on the X chromosome; all other known blood group genes are carried on autosomes. This is proving to be a valuable tool in the study of human genetics.

## Blood Groups and Human Genetics<sup>196</sup>

Reference has been made in the preceding pages to the inheritance of the blood groups. Provided potent antisera are used and meticulous technique is employed, their value for studies of human genetics is apparent. As sharply distinguishable "fixed" characteristics of an individual, they serve as important markers of chromosomes and are useful in the study of linkage, the phenomenon whereby characters representing genes carried on the same chromosome travel together in inheritance. The only autosomal linkage groups so far disclosed in man all involve blood groups (Lu and Sc, Rh and elliptical cells, etc).<sup>68</sup> Many studies of autosomal linkage are in progress.<sup>201</sup> The X-linked blood group system, Xg, mentioned above, was found in one family in association with the gene for hemophilia B and that for color blindness.<sup>165</sup> Such a finding permits investigation of the linear order in which these three loci are arranged on the sex chromosome.

The blood groups also permit recognition of mosaicism<sup>189</sup> and of blood chimeras. These have been observed by study of the blood of nonidentical twins. In certain instances it was shown that only some of the red cell precursors were directly inherited, the rest having been acquired as in utero grafts of migrant embryonic cells from the opposite twin. Grafting of leukocytes took place as well.<sup>68, 153</sup> Chimerism has also been demonstrated following successful bone marrow transplantation.<sup>162</sup>

## Medicolegal Applications

Race and Sanger made the conservative estimate that more than a million different kinds of blood can now be distinguished.<sup>68</sup> Because of this individuality, blood groups can be applied to various problems of identity, parentage, and paternity. In addition the ABO and Lewis antigens of secretors (page 452) can be used in the examination of dried stains of saliva, seminal stains, plasma, and muscle extracts.

The reliability of blood typing for the exclusion of the possibility of parentage has been convincingly demonstrated and such tests have been accepted as decisive in many courts.<sup>68,213</sup> The routine use of the ABO, MN, and Rh-Hr systems for such purposes has been approved<sup>159</sup> and in the hands of qualified experts the use of other blood grouping systems is also feasible. Even when only the three above-mentioned blood groups of the mother, child, and alleged father are known, it is possible to exonerate 51% of all men wrongfully accused of paternity.<sup>211</sup> Blood groups can also be used to sort out babies accidentally interchanged in maternity wards and to decide whether twins are monovular or biovular.

In applying these procedures, however, thorough familiarity with the methodology involved is essential, as the consequence of errors obviously may be harmful for all concerned.<sup>305, 222</sup>

#### Application to Anthropology— The Ethnologic Distribution of Blood Groups

The blood groups are especially fitted to throw light on the moderately remote as well as the recent origins of man. Mourant<sup>187</sup> and Boyd,<sup>147</sup> in particular, have directed their attention to this topic. The utility of the blood groups has been limited largely by the sporadic character of many investigations and, in particular, by the scarcity of studies that encompass a whole country similar to those made in Sweden.<sup>188</sup> The value of the blood group characters is especially great in the study of isolated population groups, as in Africa. A number of blood group characters are largely confined to Africans, namely, R<sup>o</sup> (Dce) and V of the Rh system; H<sub>e</sub>, H<sub>u</sub>, and S<sup>u</sup> of the MNSs system; the silent Fy of the Duffy system; and Js<sup>a</sup> (Sutter)<sup>188</sup> As previously mentioned, the Diego antigen is found almost exclusively in Mongoloid peoples. Natural selection or genetic drift is thought to be the chief mechanism responsible for such differences.

There is a marked difference in blood

group frequencies between the peoples of eastern Asia and the aboriginal peoples of America. Polynesians most closely resemble American Indians and Eskimos, although this does not prove that the ancestral Polynesians came from America.<sup>188</sup> They differ from the American Indians and Eskimos in that there is a high frequency of M in the latter peoples, a characteristic shared with the Lamuts of north central Asia.

Defining race as a population that differs significantly from other human populations in regard to the frequency of one or more of the genes it possesses, Boyd divided the races of man into five main categories: European, African, Asian, American, and Pacific.<sup>148</sup> The European group was further subdivided into five groups: (1) Early Europeans, possessing, in particular, the highest incidence of the Rh-negative gene and no B; (2) Lapps, with the highest incidence of N in Europe, high Fy<sup>a</sup>, very low B, very high A<sub>2</sub>, and infrequent Rh-negative gene; (3) Northwest Europeans; (4) Eastern and Central Europeans; and (5) Mediterraneans, variations ranging from the findings characteristic of the Lapps to the strikingly high frequency of the Rh-negative gene in the Basques. Among the Vedda, the aboriginal inhabitants of Ceylon, there is a high frequency of B and very low frequency of the A genes.<sup>216</sup> Interesting differences in abnormal hemoglobins have also been found (Chapter 24). Group B is high in Chinese and in Asiatic Indians; A is high among the Eskimos.<sup>194</sup> The highest incidence of the rare genes R<sup>a</sup> and N<sup>2</sup> is in Chinese; A<sup>2</sup>, r, K, and R<sup>1w</sup> are virtually absent.<sup>222</sup> Among the white populations of the United States, group A is almost as common as O.<sup>172</sup> In Negroes, A is less frequent than O<sup>219</sup>; R<sup>o</sup> is very high.<sup>222</sup> The frequency of R<sup>a</sup> (CDE) is higher among American Indians than among any other peoples of the world; the r gene is completely absent. Reference has been made already to the high incidence of M among American Indians and of the Diego factor, which is nearly or completely absent in Europeans. In addition, Indians from the United States and Canada are predominantly group A and O,

and almost completely lack B. Among the Pacific peoples, Polynesians differ from Indonesians and Melanesians in that they have a higher incidence of M. The Australian aborigines are characterized by high frequencies of A<sub>1</sub> and a total absence of B, Rh-negative gene, and Lu<sup>a</sup>.

With the intense interest in this topic and the availability of other genetic markers, knowledge and understanding should grow. Computers have been invented none too soon.

## Methods of Blood Typing

In the attractive style in which their monograph<sup>68</sup> is written, Race and Sanger point out, "Blood group tests need some delicacy of hand and a great deal of concentration of mind. The importance of placing the right serum and the right cells in the right tube and of correctly recording the results is almost too obvious to mention. Yet to achieve accuracy a long apprenticeship in error seems necessary. A friendly but silent atmosphere is essential. Given silence, there is still danger of the mind wandering; this it must not be allowed to do, however routine the tests may be, however primrose the alternative paths." One wonders how often failure to attend to this admonition has resulted in the untimely death of the recipient of a blood transfusion.

Blood typing and matching require red corpuscles, serum or plasma, and antisera of high activity. The simplest procedure is to obtain a suspension of red corpuscles by placing one drop of blood from the finger into 3 ml of physiologic solution of sodium chloride or by making such a dilution in a white cell counting pipet; or, better still, venous blood is obtained, 0.5 ml of this blood is expelled into a test tube containing about 10 ml of physiologic sodium chloride solution, the tube is gently shaken to wash the cells, the supernatant fluid is poured off after centrifugation, and a fresh saline solution is added to make a 2 or 3% suspension.

Red cell concentrations greater than 2 or 3% should be avoided; otherwise the cells may absorb all the agglutinins present in

weak or diluted sera and fail to agglutinate with such sera. If agglutination does occur, it may be weaker or may develop more slowly than usual. Until the test is carried out, however, the red cells should be kept in concentrated form since they retain their sensitivity better in this way.

Typing sera are usually obtained from donors deliberately immunized against blood group factors. Testing sera should be inactivated before use; when they are fresh, complement is present and hemolysis may occur. Then the red corpuscles may disappear before agglutination can be observed. It is also noteworthy that testing sera deteriorate; therefore it is necessary to test the contents of each new vial against known cell suspensions.

There are two major classes of typing sera: those capable of agglutinating red cells suspended in saline solution (saline or *complete* agglutinins) and those incapable of doing so (*incomplete* agglutinins). Complete (saline) agglutinins were at one time considered to be "bivalent" and incomplete antisera were considered to be "univalent." It is now known that "incomplete" antibodies are also structurally bivalent, but are probably incapable of bridging the gap created between cells by repulsive electrostatic forces (Chapter 27).

Red cell antigens readily detected in saline solution at room temperature include those belonging to the ABH, Lewis, MN, P, and Lutheran systems (Table 11-10). A simple method for the detection of A and B antigens, for instance, is as follows:

One drop of anti-B serum is placed on the left side of a glass slide and one drop of anti-A serum on the right side. One drop of unknown cell suspension is mixed with each of these sera and the slide is then tilted back and forth for three to five minutes. The drops may then be covered with coverslips to facilitate examination under the microscope. Clumping that is visible with the naked eye occurs in accordance with the kinds of antigens found in the red cells.

A more satisfactory procedure is carried out in small test tubes (inside diameter, 7

**Table 11-10. Optimal Conditions for Erythrocyte Antigen-Antibody Reactions**

<i>Blood Group System</i>	<i>First Choice</i>	<i>Second Choice</i>
ABO	20° C saline	14°-16° C saline
Lewis	Antiglobulin ficin-antiglobulin enzyme-antiglobulin	14°-16° C saline
Ii	4°-16° C saline	4°-16° C enzyme
Rh Hr	Antiglobulin	Proteolytic enzymes, enzyme-antiglobulin
Mn	20° C saline	14°-16° C saline
Ss	Antiglobulin	Albumin-layer
P	14°-16° C saline	20° C saline
Kell	Antiglobulin	Albumin layer
Lutheran	20° C saline	Antiglobulin
Kidd	Antiglobulin	Enzyme-antiglobulin
Duffy	Antiglobulin	Albumin layer

From Moore et al.<sup>241</sup> courtesy of the authors and the Hunter Rose Company

mm). One drop each of unknown cell suspension, saline solution, and testing serum is mixed in such a tube. Blood suspensions of known groups should be prepared at the same time to serve as controls. The tubes are centrifuged at about 2000 rpm for about one minute. They are then replaced in a rack, which is gently shaken. When agglutination has not occurred, the sediment of packed red cells at the bottom of the tube can be shaken up into an even suspension. Positive reactions are indicated by the persistence of clumps of red cells the size of which depends on the intensity of the reaction. It is desirable to examine the contents of each tube microscopically. If clumping occurs only with the anti-B serum, the red corpuscles must contain B agglutinin; if clumping occurs only with the anti-A serum, the red corpuscles must have A agglutinin; if clumping occurs with both sera, the red corpuscles must have A and B agglutinogens; and if no clumping whatever occurs, the red cells must be group O.

Since "incomplete" antibodies cannot be detected readily with saline techniques, special procedures have been developed for their demonstration. The tests are of two basic types:

#### 1. ANTIGLOBULIN OR COOMBS' TEST (see

Fig. 27-1). The direct antiglobulin test<sup>231</sup> utilizes a heterologous IgM antihuman gammaglobulin serum to agglutinate cells already coated with "incomplete" (IgG) antibodies in vivo. For purposes of red cell typing, however, the "indirect" antiglobulin test is more useful. In this modification, red cells are first incubated with a battery of specific antisera (if red cell typing is to be done) or a battery of known red cell types is incubated with an unknown serum (if the specificity of an antiserum is to be determined). After cells and antisera have had a chance to combine, the red cells are washed and are then reacted with heterologous antiglobulin sera as in the direct antiglobulin test. Agglutination indicates the presence of antibodies on the red cells.

The indirect antiglobulin test is frequently carried out with red cells that have been treated with proteolytic enzymes<sup>229</sup> such as papain, ficin, and bromelain. This process modifies the red cell envelope in such a way as to permit greater binding or more avid binding of incomplete antibodies. The use of such agents has greatly increased the sensitivity of tests for incomplete antibodies.<sup>233</sup>

In addition to its usefulness in the study of Rh antibodies, the antiglobulin test serves to demonstrate the Kell, Kidd, and Duffy systems of blood groups (Table 11-10) and

is important in the study of hemolytic anemias (Chapter 27). A great variety of techniques have been described for demonstrating agglutination.<sup>68</sup> Deserving special mention is the slanted capillary tube method which some workers have found very satisfactory for routine Rh testing.<sup>232,244</sup>

**2. PROCEDURES DIMINISHING REPULSIVE ELECTROSTATIC FORCES BETWEEN CELLS.** The reaction of incomplete antibodies with red cells may also be detected by a variety of procedures that diminish the repulsive electrostatic forces between cells and allow their approximation to such an extent that incomplete (IgG) antibodies are capable of bridging the gap between them.<sup>26,244</sup> These techniques include (a) the addition of albumin and other colloids that are presumably capable of diminishing the dielectric constant of the medium (Chapter 27); (b) the enzymatic treatment of red cells that apparently reduces the repulsive force between red cells by removal of charged surface structures, principally sialic acid residues; and (c) the reduction of the ionic strength of the medium by substituting isotonic sucrose for saline. The last method is particularly useful in automated systems.<sup>248</sup>

### Tests of Compatibility

In selecting compatible blood a minimum of three major procedures must be carried out: first, the recipient's ABO and Rh groups must be determined, as already outlined; then a compatible donor blood must be selected; and, finally, donor red cells must be cross-matched against the recipient's serum

### Cross-Match Procedure

The cross-match procedure is essential for several reasons:<sup>56</sup> (1) either donor or recipient may have been wrongly grouped; sometimes there may be subgroup differences, as when the donor is A<sub>1</sub>, but the recipient is A<sub>2</sub>, with anti-A<sub>1</sub> antibodies in his serum; (2) the recipient may carry other "natural" antibodies such as anti-P<sub>1</sub>, anti-Le<sup>a</sup>, anti-Le<sup>b</sup>, or anti-H; (3) to detect anti-Rh antibodies in Rh-

negative recipients or incompatibility due to anti-rh', anti-hr', etc; (4) to detect reactions due to other isoantibodies such as anti-K, anti-Fy<sup>a</sup>, and others.

The cross-match procedure consists of: (1) Reacting recipient serum against donor cells suspended in saline solution (page 469). (2) Testing recipient serum against donor cells in the indirect antiglobulin test (page 470). Since weak antibodies in the recipient's serum may not react with incompatible donor cells in their second or third week of storage, the recipient's serum must also be tested against a well-preserved sample representative of all clinically important antigens. (3) An enzyme test. This is included by some laboratories for the detection of Rh antibodies not reacting by the indirect antiglobulin test.<sup>244</sup>

### Sources of Error and Confusion

Confusion in typing and matching may occur as the result of pseudoagglutination or autoagglutination.

*Pseudoagglutination* refers to rouleau formation and clumping that occur when the sedimentation rate of the blood is accelerated. This is most likely to cause confusion when the slide method of typing is employed and usually disappears when pressure is exerted on the coverslip or when saline solution is added. Since the patient's serum, and particularly his plasma, contain the factors causing rapid sedimentation of red corpuscles, pseudoagglutination may cause difficulty when the patient's serum is cross-matched against the blood of prospective donors. Dilution of the serum with one or two volumes of saline solution abolishes pseudoagglutination.

*Autoagglutination* refers to the agglutination of the red cells of an individual by his own serum or plasma. Its most troublesome form is cold hemagglutination (Chapter 27). Although this phenomenon, like group-specific isoagglutination, may persist in spite of considerable dilution, it occurs usually at low temperatures, whereas specific isoagglutination is little affected by changes of temperature from 0° to 37° C. Consequently, if the test is performed at 37° C, particularly

with red cells washed with normal saline solution at this temperature, autoagglutination can be prevented.

If in blood grouping an "AB reaction" is obtained, a control test of the patient's cells in his own serum should be set up. If the reaction has been caused by autoagglutinins, agglutination will take place under these circumstances as well.

Autoagglutinins may cause confusion when the patient's serum is cross-matched against the blood of prospective donors. They can often be removed by absorbing the patient's serum with his own cells at 0° to 5°C.

Erythrocytes from old blood samples may sometimes be agglutinated by most sera irrespective of the blood groups found on those cells. This phenomenon of *panagglutination* may, therefore, be a cause of error in blood typing. It has been shown that this "Heubener-Thomsen-Friedenreich phenomenon" is due to the fact that filtrates of certain bacteria contain an enzyme that acts on human red cells, changing a latent antigen to an active one ("T-antigen"). A similar phenomenon has been described as occurring transiently *in vivo*.<sup>217</sup>

Irregular and atypical isoagglutinins occurring naturally, such as those reacting with subgroups of A and the agglutinogens P and M, generally give much weaker reactions than typical isoagglutinins and react only exceptionally at 37° C. They may therefore be confused with cold autohemagglutinins. Other irregular isoagglutinins resulting from isoimmunization, especially the Rh-Hr antibodies, generally react at body temperature and may be quite potent, giving rise to dangerous hemolytic transfusion reactions. Special procedures are required to detect some of these.

## Collection and Preservation of Blood

The technology of collection and preservation of blood for transfusion has been greatly improved. Plastic collecting bags with one-piece tubes and disposable needles have replaced glass containers, vacuum bottles, and

rubber tubing with their potential hazards. Various safeguards have been introduced in blood banking and transfusion practices.<sup>211,215</sup> Double and quadruple bags are available for the plasmapheresis of donor blood in a closed system, thereby eliminating the most common cause of bacterial contamination. In addition, the nonwettable surface of the plastic minimizes the danger of clot formation. Pilot tube samples can be made an integral part of the plastic tubing so that the danger of error from mix-up of tubes can be avoided. The blood preservative is prepared and autoclaved in the bag at the time of manufacture and quality control and pyrogen testing can be carried out efficiently and safely by the manufacturer.

### Red Cell Storage Lesions

When blood is stored in liquid medium, a series of readily discernible biochemical changes that influence the viability of stored cells occurs.

1. The first important change consists of a sharp drop in 2,3-diphosphoglyceric acid (2,3DPG) levels, which may reach 20% of normal within five days.<sup>226,230</sup> The level of 2,3DPG within the red cell is an important determinant of hemoglobin function:<sup>236</sup> falling 2,3DPG levels are accompanied by a shift to the left in the oxygen dissociation curve, indicating a higher oxygen affinity of hemoglobin (Chapter 3). This increased oxygen affinity of stored blood may be of critical clinical importance; a seriously ill patient receiving large doses of blood with a high affinity for oxygen may look pink, but his tissues may not benefit because the hemoglobin of 2,3DPG-depleted red cells does not unload oxygen as readily as the hemoglobin of normal cells. Transfused red cells totally depleted of 2,3DPG can regain half their normal level within 24 hours or less,<sup>230,242</sup> but this reconditioning may not be rapid enough for the seriously ill patient.

2. The concentration of ATP falls much more gradually than does the level of 2,3DPG, while the concentration of ADP and AMP first rises but then decreases as AMP

is irreversibly deaminated to IMP, IMP being subsequently catabolized to hypoxanthine.<sup>227</sup>

3. As the concentration of ATP falls, red cells lose the ability to phosphorylate glucose, and the sodium-potassium pump breaks down. Potassium leaks from the cell and sodium gains entry into the cell. As a result the osmotic fragility of stored red cells is increased and some undergo spontaneous lysis.

When stored blood is reinfused, the most damaged cells are removed within the first 24 hours, whereas the remainder appear to survive normally or almost normally.<sup>236,251</sup> When freshly collected ACD blood is transfused, an average of 5% of the cells is lost during the first 48 hours post-transfusion. Approximately 90% of the red corpuscles survive in usable form after 14 days' storage but only 70% remain after 24 days. A 70% long-term survival rate usually is considered satisfactory for transfusion purposes.

The loss of organic phosphates is one of the most important aspects of the storage lesion (see above); therefore attempts have been made to preserve the concentration of organic phosphates within the red cell. Thus the addition of adenine at the beginning of storage prevents the loss of ATP and enhances the *in vitro* preservation of red cells considerably.<sup>241,250</sup> The addition of adenine is not helpful once the red cells have lost a considerable portion of their ATP, since they have then lost their ability to phosphorylate glucose, incorporate adenine into adenine nucleotides, or phosphorylate AMP and ADP to ATP. However, if inosine is added to blood, even after several weeks of storage, a prompt boost in 2,3DPG and ATP levels occurs,<sup>230,250</sup> because the phosphorolysis of inosine yields ribose-1-phosphate which in turn can be metabolized to high-energy phosphates. There is a concomitant improvement in the storage viability of red cells. Unfortunately the potential toxic effects of intravenously administered inosine may limit its usefulness; effective amounts impose a high purine load on the kidneys and dangerous hyperuricemia may develop.

## Anticoagulants

Several types of anticoagulants are available for liquid storage of red cells. *Acid citrate dextrose* (ACD) solution (NIH Formula A) contains 7.3 g citric acid, 22.0 g sodium citrate, and 24.5 g glucose per liter of solution. Of ACD Formula A, 15 ml must be available for every 100 ml of blood collected. ACD Formula B is somewhat more hypotonic: 1 liter of solution contains 4.4 g citric acid, 13.2 g sodium citrate, and 14.7 g of glucose; 25 ml of ACD Formula B must be available per 100 ml of blood.

Better initial survival as well as a longer red cell "half-life" ( $t_{1/2}$ ) were observed when a citrate-phosphate dextrose (CPD) preservative was used.<sup>228,236</sup> CPD represents only a slight modification of the basic ACD formula and contains 3.2 g citric acid, 25.8 g sodium citrate, 25.0 g of glucose, and 2.18 g of  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  per liter of solution. Fourteen milliliters of CPD solution must be available per 100 ml of blood collected. The net loss of intracellular potassium and the net gain in plasma potassium were found to be significantly less in CPD than in ACD blood, and plasma potassium levels were lower throughout storage,<sup>237</sup> but the formulaic inorganic phosphate of CPD results in higher plasma phosphate levels than in ACD blood. When CPD is used as a preservative the blood-preservative mixture is somewhat more alkaline than when ACD is used and this results in better 2,3DPG preservation.<sup>230</sup>

*Heparin* has also been used as a preservative, but when the blood is cooled to 4° C there is a marked rise in the pH and consequently a rapid loss of red cell ATP. Heparinized blood must therefore be used within 48 hours of collection. Heparinized blood is particularly popular for the priming of extracorporeal circuits, but, for these purposes, glucose and low molecular-weight dextran may be even better.<sup>234</sup>

## Frozen Red Cells

In the preservation of blood for long periods, measured in years, glycerol has been

found to protect cells against internal crystal formation at temperatures of  $-80^{\circ}\text{C}$  and lower.<sup>255,256</sup> When needed the blood can be thawed, warmed, and reconstituted with only a 20% loss of red cells. The freezing of red cells in liquid nitrogen also holds promise as a method of preservation.<sup>252</sup>

Frozen red cells have unique applications because of their almost unlimited shelf life, the superior preservation of organic phosphates such as 2,3DPG and ATP, and the extent to which leukocytes, platelets, and plasma are removed in the various washing procedures required during deglycerolization.<sup>255,257</sup> The incidence of hepatitis appears to be markedly decreased.<sup>251</sup> The clinical usefulness and safety of frozen blood have been tested in various clinical situations, including its use in the emergency therapy of combat casualties.<sup>255</sup> A particularly useful role for frozen blood appears to be the establishment of a "rare blood" bank for the stockpiling of unusual blood types.<sup>259</sup>

## Blood Transfusion

The transfusion of blood from one dog to another was achieved successfully in the 17th century,<sup>270</sup> but the transfusion of blood from man to man, with rare exceptions,<sup>271</sup> was not accomplished until the mysteries of serologic incompatibility were disclosed, as discussed above. In the earlier attempts, blood was collected in paraffin-lined containers, or "direct" transfusions were given, in which communications were made between the vessels of the donor and those of the recipient. Sodium citrate was introduced as an anticoagulant for blood in 1914 and two decades later it was shown that cadaver blood, because of the fibrinolysis which takes place after death, could be used for transfusion without mixing it with anticoagulants.<sup>284</sup> It was not, however, until the concept of blood banks was introduced and the exigencies of World War II stimulated the investigation of methods for blood preservation<sup>278</sup> that blood became readily available and blood transfusion became popular. This, however, has not been an unmixed blessing, for familiarity has bred

thoughtlessness and now, in civilian practice at least, blood transfusion is carried out all too frequently when it is not required and without due consideration of the risks involved. It was found in one study that 27% of multiple and 60% of single unit transfusions were not indicated!<sup>261</sup>

### Indications

Blood transfusion must be regarded as a rather dangerous and potentially lethal form of therapy and clear indications for its use must therefore exist. The physician must consciously and deliberately weigh the potential benefits against the known risks. In chemotherapy the ratio of the two is known as the therapeutic index and similar concepts are well applied to the use of blood and blood products.

When transfusion seems indicated, the physician must also decide whether the patient needs whole blood or blood components and how much needs to be given.

**WHOLE BLOOD.** By far the most important use of whole blood is for the restoration of an adequate blood volume after hemorrhage or trauma. In such situations the restoration of an adequate volume is usually more important than an adequate red cell mass; therefore whole blood transfusion has no equal. Saline, plasma, and "plasma expanders," such as low molecular-weight dextran, may be useful stopgap measures until the typing and matching have been done. However, the use of dextran may give rise to difficulties in blood matching; therefore an adequate blood sample should be removed for matching before dextran has been given.

Accurate assessment of the amount of blood lost is difficult, although an estimate based on the blood pressure, pulse rate, and the patient's general appearance can often be made. The signs and symptoms of severe blood loss include pallor, vasoconstriction, sweating, thirst, air hunger, and restlessness. When such manifestations of oxygen want are present, transfusion is always mandatory. When there is evidence of acute ongoing



hemorrhage, however, it is unnecessary to wait for the development of these extreme signs and symptoms, as many adults may lose as much as 1500 ml of blood without showing such clinical signs, so long as they remain in the horizontal position.<sup>274</sup> Under such circumstances the blood pressure and other clinical parameters should be observed with the patient in the "tilted" as well as in the horizontal position. Acute blood loss in excess of 1500 ml produces clinical shock in most persons.

If the systolic blood pressure is less than 100 mm of mercury, the blood volume is probably less than 70% of normal, but it is dangerous to rely on this sign alone when the patient's pre-hemorrhage pressure is not known. Thus a systolic pressure of 120 mm of mercury may be very significant in a hypertensive patient whose usual systolic pressure is 200 mm of mercury. Similarly the pulse rate is by itself an unreliable guide, although in an actively bleeding patient a persistent pulse rate of 100 or more probably indicates blood loss in excess of 20%. Estimation of the hemoglobin level or hematocrit value may be misleading shortly after an acute hemorrhage, since compensatory vasoconstriction will keep these at normal levels until the intravascular volume has been re-expanded with fluids from extravascular sources. This usually occurs between three and six hours after hemorrhage, and a rapidly falling hemoglobin level during this period indicates blood loss of serious proportions. The skillful physician will, of course, not allow these signs of major blood loss to develop.

The loss of blood during surgical procedures is partly a reflection of the skill and care of the operator and, to a lesser extent, the type of operation. The common practice of replacing all blood losses by transfusion is totally unjustified. The loss of 500 ml of blood is well tolerated by most patients. Indeed, even patients undergoing open-heart and other major surgical procedures in which blood loss exceeds 1000 ml can be managed without blood transfusion, so long as intravascular volume is maintained with crystal-

loid solutions<sup>268</sup> or even with simple saline infusions.<sup>279</sup> When noncolloid solutions, such as buffered saline are used, the volume administered needs to be two to three times the volume of blood lost.<sup>275,279</sup>

Whole fresh blood is indispensable for exchange transfusions such as those used in the treatment of hemolytic disease of the newborn (Chapter 27). It is also most commonly used to prime the equipment for extracorporeal shunts, including renal hemodialysis units and heart-lung machines.<sup>261,282</sup> It has been suggested by some, however, that packed red cells suspended in suitable balanced salt solutions are preferable for these purposes,<sup>262</sup> while others have recommended priming extracorporeal circuits with blood substitutes such as crystalloid or colloid solutions.<sup>234,317</sup>

**PACKED RED CELLS.** Except as outlined in the previous section, most transfusions are given in order to improve the oxygen-carrying capacity of the blood. The only component of donor blood that can accomplish this is the red cell and thus, when improvement of the oxygen-carrying capacity is the objective, all other blood components are wasteful or dangerous. The plasma is needed for the preparation of various plasma fractions such as albumin, cryoprecipitate, or gamma globulin, and platelets are in high demand for patients suffering from thrombocytopenia. In addition, for improvement of the oxygen-carrying capacity the various unneeded components are potentially dangerous to the recipient; leukocytes and platelets carry transplantation antigens to which the patient may become sensitized. The resulting isoantibodies may later give rise to transfusion reactions (page 479), isoimmune leukopenia of the newborn (page 507), and rapid elimination of transfused platelets (Chapter 12). In patients undergoing hemodialysis, blood transfusion may prejudice the survival of an organ transplant in the future.

The transfusion of plasma, citrate, and electrolytes that are part of whole blood also expands the circulating blood volume and this may be poorly tolerated in patients with in-

Table 11-11. Packed Red Cell Products Prepared from Blood Collected in Plastic Containers

Product	Hematocrit (%)	Percentage of Original Unit			Method of Separation*	Permissible Storage Life at 1-6° C after Preparation
		Leukocytes	Platelets	Plasma		
Sedimented cells	65-70	100	100	30	Generally open	Not > 24 hr
Centrifuged cells†	80	100	100	15	Open	Not > 24 hr
					Closed	Up to 21 days from collection
Centrifuged cells† with buffy coat squeezed off	>90	30	<30	5-10	Open	Not > 24 hr
					Closed	Uncertain—possibly up to 10-15 days from collection
Washed cells (continuous-flow centrifuge)	As desired	<10	<10	<1	Open	Not > 3 hr
Thawed resuspended cells (continuous flow centrifuge)	As desired	0	0	<1	Open	Not > 3 hr

\* Plasma removed by breaking of container's hermetic seal (open) or into attached bag with hermetic seal unbroken (closed)

† Centrifugation permissible for blood stored for not > six days from date of collection  
From Chaplin<sup>262</sup> courtesy of the author and New England Journal of Medicine

recipient circulatory failure (page 485). In addition, plasma proteins are themselves antigenic and there have been well-documented instances of anaphylactic transfusion reactions, involving primarily anti-IgA antibodies in recipients lacking this particular immunoglobulin (page 485). Plasma also carries isohemagglutinins; multiple units of O blood given to A, B, or AB individuals could lead to the destruction of the recipient's own red cells. The protein content of plasma is inconsequential in the treatment of patients with hypoproteinemia and malnutrition for whom it is frequently prescribed, its beneficial effect on wound healing is a myth.

The available packed red cell preparations and their properties are listed in Table 11-11.<sup>262</sup> It becomes immediately apparent that most techniques efficiently reduce the amount of plasma and anticoagulant, thereby

minimizing the possibility of circulatory overload, electrolyte disturbance, and the effects of transfused isohemagglutinins. Only one of these techniques, however, reduces the concentration of leukocytes and platelets sufficiently to prevent isosensitization. In only two of these preparations is the concentration of plasma proteins reduced to a small enough fraction of the original (<1%) to reduce the incidence of transfusion hepatitis (page 486).

*Sedimented cells* are most readily available, but the recovery of plasma is inefficient. All the leukocytes and platelets remain. Since withdrawal of plasma from sedimented cells usually breaks the hermetic seal of the original container, the unit must be administered within 24 hours to avoid the risks of bacterial contamination.

*Centrifuged cells* permit the salvage of more

plasma but offer no additional advantage in regard to leukocyte and platelet sensitization and the risk of hepatitis. The plasma, however, is usually removed within a closed system and centrifuged cells may therefore be stored at 4° C for up to 21 or 28 days.<sup>285</sup>

Centrifuged blood from which the buffy coat has been squeezed off has the highest hematocrit value of any of the products and saline or balanced salt solutions generally have to be added just prior to use to reduce viscosity and facilitate transfusion. It is obvious, however, that about 30% of the original leukocytes remain behind and while this may be sufficient to reduce the incidence of reactions in weakly sensitized patients, it is ineffective in patients with a moderate or high degree of isosensitization. A more satisfactory buffy-coat-poor preparation can be obtained by centrifuging the plastic container in the inverted position and draining off the red cells from below.<sup>262</sup> When about 25% of the red cell mass is left behind less than 10% of the original white cells will be found in the final product.

*Red cells washed in saline solution* by manual techniques offer little advantage over the centrifuged cells described above. Cells washed in a continuous flow centrifuge will, however, be remarkably free of plasma, leukocytes, and platelets. This also is an ideal way to prepare washed red cells for patients suffering from PNH (Chapter 29).

The characteristics of *frozen red cells* have been discussed previously (page 474).

**USES.** 1. Packed cell transfusions are indicated for patients with certain forms of *hemolytic anemia*, especially when they are in aplastic crises. Such patients are frequently very ill and may have hemoglobin values as low as 2 to 3 g/dl. Their cardiovascular system often is on the verge of collapse and even a small transfusion may precipitate cardiac failure and death. They can be managed safely only by constant central venous pressure monitoring and by exchanging packed cells cautiously for their blood. A careful balance sheet must be kept and initially it is well to remove perhaps 10% more volume

than is replaced. Packed cells should be as fresh as possible. An anemic patient with an elevated venous pressure at the start of therapy must *never* be transfused without continuous central venous pressure monitoring and the concomitant removal of blood as necessary, since, otherwise, cardiac failure will almost inevitably become worse.<sup>265,266</sup> Digoxin and diuretics may on occasion be useful ancillary measures, but are not as reliable as an exchange transfusion.

In acquired forms of hemolytic anemia, transfused red cells may be destroyed as rapidly as are the recipient's own cells. When hemolysis is due to an antibody it may be possible to identify its specificity; in such cases it may be possible to find compatible blood (Chapter 27).

In the more chronic forms of hemolytic anemia a state of equilibrium frequently has been reached and, in such patients, blood transfusions have little to offer.

2. Packed red cells are the treatment of choice for the support of patients with *chronic hypoplastic anemias* for whom no other forms of therapy are available. Such anemias include the various aplastic anemias (Chapter 56), the anemia accompanying chronic renal disease (Chapter 19), and others. Important considerations regarding the use of blood transfusion in patients with these anemias are discussed below.

3. Patients suffering from acute and chronic leukemia, lymphomas, or other malignant disease may need packed cell transfusions, especially while undergoing therapy.

4. Transfusions have been used to suppress *endogenous erythropoiesis* in patients with sickle cell anemia<sup>283</sup> or thalassemia,<sup>260</sup> but the danger of transfusion hemosiderosis limits the usefulness of this method (see Chapters 25 and 26).

5. Patients suffering from chronic anemias due to a deficiency of vitamin B<sub>12</sub> or iron usually respond well to specific therapy and do not require transfusions. Occasionally, however, a patient when first seen will be *critically ill* with cardiac failure, angina pectoris, cerebral insufficiency, or infection, sometimes complicated by leukopenia and

thrombocytopenia. Under such circumstances transfusion may be lifesaving<sup>27,2</sup> (Chapter 15). It should be given under close supervision with constant monitoring of central venous pressure, and, when necessary, by the exchange technique, as described elsewhere.

Some physicians follow the deplorable practice of transfusing patients whenever the hemoglobin value is below an arbitrarily set figure. Similarly, the insistence of some surgeons that the hemoglobin concentration be "normal" prior to a surgical operation is a form of superstition; the concept of "chronic shock" has no foundation in fact. Blood transfusion is not a tonic nor is it a placebo. In most patients with chronic anemia of moderate severity the anemia has developed so gradually that these patients have adapted to the new hemoglobin level. Such symptoms as may be present are often due to the underlying disease of which the anemia is merely a sign. As a rule in such a patient an equilibrium between red cell production and destruction has been reached and blood transfusions only serve to alter this balance temporarily. At the same time they subject the patient to unnecessary risks.

It is true, of course, that there is a certain level of hemoglobin below which the physiologic adjustments to anemia (Chapter 13) begin to fail and symptoms such as excessive weakness, orthopnea, and even angina will develop. These symptoms can be avoided by giving blood transfusions, but, as a rule, they do not appear in adults until the volume of packed red cells has dropped below 0.25 l/l and the hemoglobin level is under 8 g/dl. Children tolerate even lower levels. At very low levels, however, the mortality from anemia becomes considerable.<sup>26b</sup>

### Mode and Route of Administration

It is most important that the label of the bag or bottle of blood be checked against the patient's name and identification numbers. If any inconsistencies appear, the blood should not be given. Under most circumstances it is not necessary to warm the blood before transfusion unless large quantities have to be given quickly.<sup>58</sup>

Blood should be given slowly at first, since this will allow for detection of unexpected transfusion reactions before the patient is seriously harmed. After the first 30 minutes, blood usually is given at the rate of 200 to 400 ml/hour in adults; in many patients without cardiorespiratory problems, rates of 500 ml/hour are perfectly safe.<sup>56</sup> Packed cells should be infused at about two thirds the rate of whole blood. In patients with incipient cardiac failure or elevated venous pressure, blood must be given much more slowly and, preferably, by exchange transfusion (page 477).

In infants and children the blood is given at a rate of 2 to 6 ml/kg of body weight/hour, depending on the condition of the child, but under most circumstances no more than 15 to 20 ml/kg are given at any one time. When blood is lost acutely, for instance, by hemorrhage, larger amounts of blood may, and frequently must, be given quickly in order to save the life of the patient.

The amount of blood required to raise the hemoglobin to a desired level can be calculated easily if one remembers that a hemoglobin concentration of 15 g/dl corresponds to a red cell volume of about 30 ml/kg of body weight. Since most packed cell preparations have a hematocrit value of about 0.66 l/l, 3 ml of packed cells/kg of body weight will raise the hemoglobin concentration by 1 g/dl.<sup>56</sup> If whole citrated blood is used (hematocrit = 0.33 l/l), twice as much volume has to be given in order to achieve the same result.

The usual site for transfusion is one of the antecubital veins of the forearm, but the superficial veins of the legs and, in infants, the scalp veins also may be used. When, for various reasons (peripheral circulatory failure, extensive skin burns, etc), veins are not accessible for transfusions, blood and other suitable fluids may be given via the bone marrow,<sup>287</sup> but the procedure is not without danger. For extreme emergencies, as in the treatment of shock resulting from a rapid decrease in blood volume when conventional methods of therapy have failed, transfusions have been given intra-arterially,<sup>280</sup> but it is doubtful whether this is ever justified, except

into the arterial stump left by a destroyed arm or leg of a person in severe shock. Intraperitoneal transfusion is a simple procedure that can be accomplished quickly and easily,<sup>288</sup> but absorption of blood is slow.

### Complications of Blood Transfusion

Transfusion reactions are conveniently classified as *immune* or *nonimmune*. The former include (1) reactions due to red cell incompatibility and (2) reactions due to leukocyte and platelet incompatibilities, as well as other forms of allergic reactions. Non-immune transfusion reactions include (1) those caused by overloading of the circulation, (2) those related, in particular, to massive transfusions, (3) transmission of infections, and (4) miscellaneous ill-effects, such as thrombophlebitis, air and fat embolism, and transfusion siderosis.

### *Immunologically Mediated Transfusion Reactions*

#### 1. Hemolytic Transfusion Reactions

Two different mechanisms cause hemolysis of mismatched blood—hemolysis may be due to rapid intravascular breakdown of transfused cells or it may be due to extravascular destruction of antibody-sensitized erythrocytes, predominantly in the reticuloendothelial system. The immunologic basis of the two types of hemolysis and the kinetics of antibody-mediated red cell destruction are discussed in Chapter 27. This section will deal predominantly with the clinical manifestations of transfusion reactions.

#### *Intravascular Hemolysis of Red Cells*

This type of transfusion reaction is most commonly associated with incompatibility in the ABO system, since anti-A and anti-B antibodies are most commonly IgM and are capable of binding complement, leading to almost instantaneous lysis of transfused cells in a sensitized individual.

The most common cause of ABO incom-

patible transfusion reaction probably is human error. Other causes include the weak  $A_2$  and  $A_2B$  agglutinogens that may be missed during typing. This problem was discussed earlier (page 472); other atypical blood groups, such as  $A_x$  (Wiener's "C"), also may be associated with hemolytic transfusion reactions.<sup>365</sup>

Occasionally severe transfusion reactions have been caused by the use of "universal donor" blood (O Rh negative), usually under emergency conditions when not enough time was available to type and cross-match compatible blood. While this blood can often be employed successfully, especially if it has low titers of anti-A and anti-B, its routine administration in emergencies cannot be recommended. In most circumstances, transfusion can be delayed for 15 or 30 minutes while the patient's ABO and Rh types are ascertained so that appropriately grouped blood can be provided, even if there is not sufficient time for a cross-match. Sometimes severe hemolytic reactions have been observed even when the anti-A agglutinin titer of the donor's blood was not unusually high.<sup>369</sup> The dangerous anti-A antibodies in such instances have shown characteristics of an "immune" type of antibody in that they agglutinated more readily at 37° C than at lower temperatures, agglutinated red cells only in serum or albumin, fixed complement and acted as hemolysins, and were relatively resistant to the addition of soluble A and B substance. Such agglutinins have been encountered particularly in persons who have been immunized with horse serum or similar agents that might have stimulated the production of such antibodies,<sup>369</sup> as well as in group O mothers immunized by type A or B infants.

When group O is used as universal donor blood, the titer of isoagglutinins must first be determined. When necessary the presence of a safe level can be established quickly by diluting the serum of the donor 1:200 and then mixing it with a suspension of appropriate red cells. If agglutination does not occur under these conditions, the blood may be regarded as safe. Additional qualifications that should be required of "safe universal" group O blood are that it be (1) truly group

O and not  $A_2$  or  $A_2B$  mistaken for O or B; (2) Rh negative; and (3) lacking atypical iso-antibodies such as anti-H, anti-M, and anti-P. With the isolation of group-specific A and B substances, it is possible to reduce the titer of antibodies in group O blood by adding these substances to the blood.<sup>342</sup>

**CLINICAL MANIFESTATIONS.** The administration of incompatible blood usually is associated with the onset of symptoms before much blood has been introduced; if the transfusion is stopped, no serious harm may result. The symptoms observed include restlessness, anxiety, flushing of the face, precordial oppression and pain, an increase in pulse and respiratory rates, generalized tingling sensations, and pain in the back and thighs. Nausea and vomiting may follow and cyanosis, shock with cold, clammy skin, coma, and a failing pulse may develop. A chill, followed by a rise of temperature to 105° F or higher, and even delirium may ensue. Leukopenia is followed by leukocytosis.

Not infrequently a *hemorrhagic tendency* develops immediately after the transfusion of incompatible blood and blood may ooze from the site of transfusion, from mucous membranes,<sup>321 339</sup> or from the operative site if such exists. Fibrinogen and other labile clotting factors are usually found to be depleted, probably as the result of fibrin formation after liberation of thromboplastic substances from red cells hemolyzed intravascularly.<sup>330</sup> Thrombocytopenia may be present as well.

*Hemoglobinemia* can be detected by examining the blood plasma without delay. Hemoglobinuria and jaundice, as well as oliguria or even anuria, may follow, but hemoglobin may be present only in the first urine specimen passed after the transfusion. Signs of *renal involvement* may develop rapidly and subside in large measure after 24 hours, only to be followed by a progressing state of uremia; or the signs may seem to be insignificant at first and yet are followed by evidence of severe renal impairment. The latter may be succeeded after several days by diuresis and recovery may then occur if proper care has been given (page 483), or uremia may persist.

Death has occurred as late as the seventh to nineteenth day following transfusion, while diuresis and recovery have appeared as late as the sixteenth day.<sup>317</sup>

The mechanism involved in the production of the *renal failure* has been the subject of study for many years. It is clear that hemoglobinemia per se is not the primary cause of the disorder. Injections of hemoglobin into normal animals have not produced hemoglobinuric nephrosis, but when hemoglobin was given intravenously to anesthetized, dehydrated rats, renal ischemia and failure of glomerular filtration developed and were followed by tubular destruction associated with the formation of hemoglobin casts.<sup>322</sup> Focal cortical ischemia has been described.<sup>349</sup> It seems likely that the precipitation of hemoglobin in renal tubules depends upon the functional abnormality of nephrons,<sup>331</sup> which probably is the consequence of circulatory failure associated with transfusion reactions. In addition to the peripheral vascular failure, many other variables contribute to the development of renal failure, including the amount of reduction in urine volume and increased hydrogen ion concentration of the urine. The latter favors the precipitation of hemoglobin in the renal tubules, thereby producing mechanical blockage.

### *Extravascular Hemolysis of Red Cells*

Rh incompatibility is by far the most common cause of transfusion reactions due to extravascular hemolysis. Fortunately anti-Rh antibodies do not occur spontaneously and do not develop in significant titer in every Rh-negative person exposed to Rh-positive blood (Chapter 27).

Of the various components of the Rh-Hr system, anti- $R_h$  (D) is by far the most common antibody causing reactions and anti- $R_h$  plus anti-rh' (CD) is another frequent cause. Anti-hr' (c), anti-rh'' (E), and anti- $R_h$  plus anti-rh'' (anti-DE) are not rare, but other antibodies involving this system are found in less than 1% of the patients having transfusion reactions due to extravascular hemolysis.

In an earlier section, certain Rh alleles for

**Table 11-12. Approximate Relative Frequency of Rh Antibodies**

Fisher Terminology	Wiener Terminology	Approximate Percentage Frequency <sup>56, 63, 187</sup>
Anti-D	Anti-Rh <sub>0</sub>	40-70
Anti-D + anti-C	Anti-Rh <sub>1</sub>	8-30
Anti-c	Anti-hr'	1-2
Anti-E	Anti-rh''	1-10
Anti-D + anti-E	Anti Rh <sub>2</sub>	2-4
Anti-C	Anti rh'	<1
Anti-C*	Anti rh''	<1
Anti-e	Anti-hr''	*

< Indicates less than

\*Some examples reported

which specific antisera have not been found were mentioned (page 460). The antibodies for these antigens seem to be inseparable from certain anti-Rh<sub>0</sub> (D), anti-rh' (C), anti-hr' (c), and anti-rh'' (E) sera,<sup>68</sup> respectively. Of these, the Rh<sub>0</sub> variants (D<sup>u</sup>) appear to be quite important because they are sufficiently antigenic to provoke an immune response, and can give rise to transfusion reactions or

erythroblastosis fetalis; yet, since Rh<sub>0</sub> (D<sup>u</sup>) gives weaker reactions than do other Rh-positive bloods, the blood of an Rh<sub>0</sub> (D<sup>u</sup>) carrier may be mistakenly considered to be Rh negative.<sup>324</sup> Routine use of the antiglobulin technique in typing for Rh antigens can prevent this error. Among the other Rh alleles, anti-rh<sup>w1</sup> (C<sup>w</sup>) and anti-rh<sup>w2</sup> (E<sup>w</sup>) have been known to cause erythroblastosis fetalis.<sup>319</sup>

Table 11-12 indicates that antibodies to Rh-negative blood are not so rare and this should emphasize the fact that the transfusion of Rh-positive patients with Rh-negative blood is potentially dangerous. In a number of patients, transfusion reactions due to this cause have been observed.<sup>68</sup> These have usually been so mild in degree as to be passed off as pyrogenic reactions, but death due to the administration of Rh-negative blood has been reported.

In Table 11-13 the clinical importance of the various blood groups in causing sensitization and transfusion reactions or hemolytic disease of the newborn is indicated. It

**Table 11-13. Blood Groups: Clinical Importance and Sensitivity to Agglutination**

System	Isoantibodies		Components Important in Sensitivity Reactions			Sensitivity to Agglutination by Various Techniques			
	Natural	Isoimmune	Frequently	Rarely	Unknown	Saline	Albumin	Enzymes	Antiglobulin
ABO	Regularly present*	Common	A, B	—	A <sub>1</sub> B <sub>1</sub> , O	++	+	+	++
Rh Hr	Never	Common	D, c, E	C C <sup>w</sup> D <sup>u</sup> E <sup>w</sup> e	†	—	+	++	++
Kell	None	Occasional	K†	k	—	—	±	±	++
Kidd	None	Rarely	—	Jk <sup>a</sup>	Jk <sup>b</sup>	±	—	—	++
{M N	Rare	Rare	—	M N	—	++	+	—	+
{S-s	Rare	Rare	—	S, s U	—	±	±	+	++
				M <sup>a</sup> V <sub>a</sub>	—				
Duffy	None	Rarely	—	Fy <sup>a</sup>	—	±	—	—	++
Lewis	Infrequent	V rarely	—	Le <sup>a</sup>	Le <sup>b</sup>	++	+	++	+
Lutheran	None	1 case	—	Lu <sup>b</sup>	Lu <sup>c</sup>	++	+	+	±
P	Regularly	—	—	Anti-P <sub>1</sub>	—	++	+	+	±

The blood group systems have been listed in the order of their clinical importance. The order of genetic importance is MNSs, Rh-Hr, ABO and Kidd. Those most readily useful in medicolegal work are ABO, MN and Rh-Hr; other blood factors are rarely or never used.

\*Except during neonatal period

†By no means comparable in frequency with ABO or Rh Hr system

Key to sensitivity ++ indicates preferred technique, + means almost always positive ± may be positive or negative, — almost always negative

is noteworthy how important the Kell factor is from the standpoint of sensitization. Fatal transfusion reactions have been observed.<sup>352</sup> It may be added that a number of the "private" blood group antigens (page 467) also have been associated with hemolytic disease.<sup>68</sup>

The clinical manifestations of extravascular hemolysis (Chapter 20) are less spectacular than those of intravascular red cell destruction. Fever and chills develop frequently but may be delayed in onset for an hour or so. It has been suggested that these febrile reactions are somehow related to splenic sequestration.<sup>56</sup> Acute renal failure is not characteristic of extravascular hemolysis.

#### *Hemolytic Transfusion Reactions in the Absence of Demonstrable Incompatibility*

Several patients have been described in whom transfused red cells were rapidly destroyed even though no antibody was demonstrable at the time of transfusion.<sup>350</sup> In a number of patients, antibody was discovered some days after transfusion or was known to have been present before transfusion.<sup>325</sup> In one subject, however, exhaustive studies failed to reveal an antibody.<sup>371</sup> It may well be that gradually increasing transfusion requirements in patients receiving very many transfusions are due to undetected sensitization. It would be of interest to study such patients by a double-labeling technique<sup>372</sup> in which the suspected incompatible cells would be labeled with <sup>51</sup>Cr and, as a control, the recipient's own cells would be labeled with <sup>42</sup>P.

#### *Delayed Transfusion Reactions*

Delayed transfusion reactions are most commonly due to isoantibodies, such as those directed against Rh-Hr antigens, and may not appear until 2 to 14 days after the transfusion of blood. Two explanations have been offered for this phenomenon.<sup>56</sup> Either antibody is present in the recipient's serum but went undetected because of its low titer, or anti-

body is not detectable by any available method but shows a rapid rise subsequently because the transfusion has triggered a secondary (anamnestic) response. In either instance, hemolysis does not become evident until antibody is produced in sufficient quantity; it may then be very abrupt in onset. To the unwary the eventual development of a positive reaction to a direct Coombs' test in such patients may suggest a diagnosis of autoimmune hemolytic anemia.<sup>306</sup>

#### *Laboratory Investigation of Hemolytic Transfusion Reactions*

Laboratory tests utilized in the investigation of hemolytic transfusion reactions include: (1) those demonstrating red cell destruction, (2) serologic tests to determine the cause of the transfusion reaction, and (3) tests for determining the presence of disseminated intravascular coagulation (Chapter 38). The appropriate investigations have been summarized in Table 11-14.

Hemolysis is evidenced by elevated plasma hemoglobin levels, hemoglobinuria, and hyperbilirubinemia assessed by the indirect technique. Reduced haptoglobin levels indicate intravascular hemolysis; determination of these levels is particularly useful when the

**Table 11-14. Tests to be Performed When Hemolytic Transfusion Reactions Are Suspected**

- 
- |   |  |
|---|--|
| A | Demonstrating red cell destruction               |
|   | Increased plasma hemoglobin                      |
|   | Hemoglobinuria                                   |
|   | Reduced haptoglobin levels                       |
|   | Methemalbuminemia                                |
|   | Hyperbilirubinemia                               |
|   | Differential agglutination for red cell survival |
| B | Tests for intravascular coagulation (Chapter 38) |
| C | Serologic studies                                |
|   | Complete retyping of patient and donor blood     |
|   | Direct Coombs' test on recipient's cells         |
|   | Indirect Coombs' test                            |
|   | 1 Donor cells and recipient's serum              |
|   | 2 Recipient's cells and donor serum              |
| D | Others   |
|   | Aerobic and anaerobic culture of donor blood     |
|   | Tests of renal function                          |
-



reactions are mild. With severe transfusion reactions, methemalbuminemia may also appear. When transfusion reactions are mild, comparison with pre-transfusion pigment values is valuable but is not always possible. It is important to draw blood carefully since artifactually induced hemolysis may make interpretation of data difficult.

Serologic tests include complete retyping of the patient's and donor's blood in an effort to identify the responsible blood group incompatibility. The cross-match should be reconfirmed. In addition, the patient's red cells should be tested for surface immunoglobulin and complement by means of the direct antiglobulin test; the patient's serum should be tested for antibodies against donor cells and the donor's serum should be tested for antibodies that may react with recipient cells.

It is also necessary to examine the administered blood for aerobic and anaerobic bacterial contamination. The urine output should be monitored carefully and the blood should be watched for the development of azotemia.

### Treatment

Since the renal complications are the most lethal, the major aim of therapy must be their prevention and treatment.

PREVENTION depends on the maintenance of renal blood flow. This is accomplished in several ways. (1) Treatment with heparin should be given when intravascular coagulation is present since it probably contributes significantly to the development of renal failure.<sup>360,363</sup> (2) The infusion of the osmotic diuretic mannitol has been recommended as a means of maintaining urine flow, glomerular filtration, and renal blood flow when oliguria is observed.<sup>295</sup> It is customary to give 20 g of mannitol as a 20% solution over a five-minute period. If no urine flow occurs, the initial injection may be repeated, but not more than 80 to 100 g should be used within a 24-hour period. If there is no improvement within 24 hours the trial should be abandoned. Mannitol is contraindicated once acute tubular necrosis has occurred. If

diuresis is induced by mannitol, adequate fluid replacement has to be provided. (3) For patients in shock,  $\alpha$ -adrenergic blocking agents such as phenoxybenzamine may be even more effective than mannitol in increasing renal blood flow,<sup>348</sup> but these experimental agents must only be given by physicians experienced in their use. In animal models, phenoxybenzamine also has been shown to have a beneficial effect in disseminated intravascular coagulation.<sup>344,345</sup> (4) Patients in shock should be treated with compatible transfusions or fluids. If there is any question as to blood compatibility, however, it is best not to give blood; plasma or other fluids should be used instead, for, as has been pointed out (page 482), erroneous conclusions may be reached when blood is examined for antibodies immediately after a transfusion reaction has occurred. (5) On the basis of animal experiments it was concluded that the precipitation of heme pigments in the kidneys is favored by an acid reaction; alkalinization of the urine (4 to 5 g of sodium bicarbonate or lactate given orally or parenterally) was recommended to prevent this. However, once the kidneys have been injured, the value of this procedure may be questioned, since renal ischemia rather than the precipitation of heme pigments in the tubules is probably the principal etiologic factor in the acute renal failure that may follow the transfusion of incompatible blood. Furthermore, the administration of excess sodium salts to patients with severe oliguria presents the double hazard of alkalosis and tetany, in addition to volume overload, congestive heart failure, and pulmonary edema.

The phase of renal insufficiency usually begins about 24 hours after the incompatible blood has been given and may last for many days. During this time it is important (1) not to flog damaged kidneys with diuretics; (2) to limit the fluid intake during this oliguric period to the insensible water loss plus measurable fluid output. Usually 400 to 500 ml per day plus a volume equal to the urinary output will suffice; however, in the presence of fever, diarrhea, vomiting, wound drainage, or other conditions resulting in fluid loss,

replacement of fluid to counteract these losses must also be made; and (3) to maintain adequate nutrition and electrolyte balance. These aims may be accomplished by (a) keeping an accurate record of fluid output and intake and maintaining fluid balance; (b) providing adequate calories by mouth or, if nausea or vomiting supervenes, by giving 50% dextrose in water continuously through a plastic catheter that empties into a large central vein; (c) replacing electrolytes that are lost. The intake should be potassium free. Repeated measurements of electrolytes should be made. Daily electrocardiograms will help in recognizing hyperkalemia. Mild sedation may be required during this period but should be avoided if possible. In critical situations, dialysis with the artificial kidney may be lifesaving.

In the final phase, during the period of tubular recovery and regeneration, there is copious diuresis, resulting in loss of salt and water that must be replaced. Replacement may necessitate the parenteral administration of fluids, but, as recovery takes place, oral administration may suffice.

## 2. Nonhemolytic (Febrile) Transfusion Reactions

Nonhemolytic febrile reactions have been reported in 1 to 20% of patients receiving transfusions, but an incidence of 3 to 4% would be a reasonable estimate for patients in most transfusion centers.<sup>358</sup> These reactions may be due to immune sensitivity to leukocytes, platelets, and various plasma constituents, or they may be due to bacterial and other pyrogens. With the advent of modern transfusion technology the latter have become quite infrequent. Often no cause whatsoever can be discovered.

A chill followed by fever may occur within an hour after the transfusion, or may be delayed for 24 hours. Headache, nausea, and vomiting may accompany this reaction. Often, however, the manifestations are mild and, if fever is the only one, the patient may not even be aware that he is having a reaction. Whatever their degree, febrile reactions usually run their course within a few hours.

*Sensitization to white cell or platelet antigens* is one of the most common causes of nonhemolytic febrile reactions,<sup>299,351,376</sup> especially in patients who have received large numbers of transfusions.<sup>308</sup> Occasionally the reaction may be severe and even life-threatening.<sup>311</sup> In one series of 269 consecutive cases of transfusion reaction, complete leukoagglutinins were found in 58% of patients suffering from chills and fever following a blood transfusion.<sup>321</sup> Such antibodies were found in only 9% of those without these symptoms. The administration of buffy-coat-poor blood may sometimes prevent these reactions (page 477).

Accurate diagnosis depends on the demonstration of antileukocyte antibodies in the recipient, but this information usually is not available when the physician is called upon to differentiate between a hemolytic and a nonhemolytic transfusion reaction. While hemolytic transfusion reactions call for the immediate cessation of the transfusion, nonhemolytic febrile responses are often well tolerated, requiring minimal supportive care usually consisting of antipyretic or antihistamine therapy, if such is not contraindicated (as in thrombocytopenia) (Chapter 35). Antihistamines are most effective if given *before* the transfusion. Unfortunately no foolproof aids to differential diagnosis are yet available and the physician will have to use his best clinical judgment in each case.

A peculiar manifestation of leukoagglutinin transfusion reactions is the appearance of *pulmonary infiltrates*.<sup>375,381</sup> This syndrome is characterized by the sudden onset of chills, fever, tachycardia, a nonproductive cough, and dyspnea. The acute symptoms last for only a few hours, but the pulmonary infiltrates persist for up to 48 hours. Eosinophilia has been noted in several of these patients.

*Plasma sensitivity* has been observed most frequently in patients with hemopoietic disorders who have received multiple transfusions.<sup>305</sup> Chills, fever, backache, pain in the legs, and intestinal peristalsis may occur. Although serologic tests reveal no incompatibility, the plasma rather than other factors may be found responsible if a few milliliters

of plasma, preferably but not necessarily from the donor whose blood is suspected, are injected into the patient. If plasma sensitivity is present, a mild reaction will occur within 30 minutes. The patient's serum should also be examined for antibodies against isologous plasma components. Plasma sensitivity is especially common in persons with paroxysmal nocturnal hemoglobinuria (Chapter 29). In these subjects, the offending plasma factor is heat-labile and can be removed by washing the red corpuscles free of plasma.

A well-defined type of "plasma sensitivity" is due to the presence of anti-IgA antibodies, particularly in patients with IgA deficiency.<sup>364</sup> Patients with this type of allergy may develop a mild reaction characterized by an erythematous rash and urticaria, but they may also develop hypotension and anaphylactic shock. They usually have no fever. Very small amounts of plasma may precipitate the reaction, which is mediated by an IgG antibody that fixes complement and has specificity for IgA. To diagnose this type of hypersensitivity it is usually necessary to demonstrate the presence of anti-IgA and the absence of IgA in the patient's serum. The reaction can be prevented by giving the patient washed red cells (page 477), which will effectively remove a large portion of the offending gamma globulin.

Precipitins reacting with low-density  $\beta$ -lipoproteins have also been found in the sera of patients receiving multiple transfusions.<sup>297</sup>

*Other allergic reactions*, characterized simply by urticaria or occasionally by swelling of lymph nodes and sore throat, eosinophilia, joint pains, and fever, occur in at least 1% of patients having blood transfusions. They may develop during the transfusion or some days later. Angioneurotic edema and asthma have been observed. Some of these reactions are due to the passive transfer of reagins to foods that the patient has eaten or the patient may have reagins for food eaten by the donor. Thus, reactions are less likely if the blood is drawn from donors who have fasted for several hours. These types of reactions respond well to epinephrine or antihistamines.

## Nonimmune Transfusion Reactions

### Circulatory Overload

The administration of excessively large quantities of blood, or smaller amounts given too rapidly ("speed reaction"), can cause circulatory failure, especially when myocardial weakness is present. Circulatory failure is often heralded by the onset of a series of short, sharp coughs, precordial and back pain, dyspnea, cyanosis, and finally a productive cough. The symptoms may develop during or up to 24 hours after transfusion, and death may result from pulmonary edema. Measurement of the patient's venous pressure before transfusion provides a useful guide to the probability of inducing cardiac failure; if the pre-transfusion venous pressure is above normal,<sup>265</sup> transfusion is likely to make the situation worse. When there is any doubt about the patient's cardiovascular status, *continuous central venous pressure monitoring* can be used to signal a dangerous increase in venous pressure. When the central venous pressure is decidedly elevated before the transfusion or rises during the transfusion, blood can only be given safely by the exchange technique (page 477). In patients with no demonstrable failure but a known history of myocardial weakness, overloading of the cardiovascular system can be avoided by transfusing blood from which most of the plasma has been removed, and by giving the blood slowly by the gravity-drip method, with the patient in a propped-up position. The rate of administration should not exceed 2 ml per kg body weight per hour and often may well be slower than this. Only when there has been acute and severe hemorrhage does blood need to be given rapidly.

### Complications Due to Massive Transfusions

With the advent of modern surgical technique and the liberal use of whole blood transfusions, two types of problems have been introduced, ie, metabolic effects result-

ing from the use of large amounts of old "bank blood" and hemorrhagic manifestations.

### Metabolic Effects

The sudden infusion of large volumes of ACD solution may cause hyperkalemia with potassium intoxication and citrate toxicity.<sup>336-340</sup> The potassium is derived from red cells and its concentration in the plasma rises to about 15 to 20 mEq/liter after 10 to 14 days' storage. Because it is metabolized rapidly, citrate rarely accumulates to the point of toxicity in adults unless citrated blood is given with great rapidity or unless the patient has impaired liver function. At citrate concentrations of about 100 mg/dl, tremors may be observed in skeletal muscles and the QT segment of the electrocardiogram is prolonged.<sup>346</sup> At still higher concentrations of citrate, cardiac arrest may develop.

With massive transfusions, ionized calcium is decreased. The intravenous administration of calcium gluconate usually prevents or eliminates the manifestations of citrate toxicity. However, hypocalcemia may not be the only ill effect produced by the infusion of large quantities of citric acid. Blood drawn in ACD solution has a pH of 6.6 on storage as the result of continued glycolysis.<sup>342</sup> Massive transfusions are followed immediately by acidosis, but, in several hours, metabolic alkalosis develops, owing to the metabolic breakdown of sodium citrate.<sup>337</sup> The advantages of CPD as an anticoagulant, as compared with those of ACD, were pointed out earlier (page 474).

### Hemorrhagic Manifestations

The rapid transfusion of large volumes of compatible stored blood may be complicated by a bleeding tendency. Neither platelets nor labile coagulation factors survive well in stored blood, and if the transfusion is equal to the blood volume of the patient, a significant dilutional effect occurs.<sup>321,329,341,384</sup> This may lead to mild thrombocytopenia and minor coagulation abnormalities.

If an incompatible transfusion which results in intravascular hemolysis is given, *diffuse intravascular coagulation* (DIC) may result (Chapter 38). Rare instances of DIC in association with extravascular hemolysis have been reported.<sup>328</sup> A rare complication of transfusion therapy is isoimmune post-transfusion thrombocytopenia.

### Transmission of Infection

*Viral hepatitis* is by far the most common and clinically important infection transmitted by transfusion. The viruses causing hepatitis appear to be hardy and highly infectious. As little as  $\frac{1}{10,000}$  of 1 ml of "icterogenic" plasma injected subcutaneously can cause clinically obvious hepatitis, and as little as  $\frac{1}{10,000,000}$  may lead to serologically detectable evidence of infection.<sup>294</sup> This is why pooled blood products are so dangerous, since virus from a single donor, though diluted, is widely distributed.

The viruses are carried by blood, plasma, albumin, fibrinogen, and AHG preparations, among others,<sup>56,379</sup> and the disease has even been transmitted with topically applied thrombin.<sup>56,379</sup> Gamma globulins prepared either by the Cohn fractionation process or by ether fractionation appear to be free of hepatitis virus.<sup>56</sup>

Two different forms of viral hepatitis that appear to be caused by antigenically different agents have been identified; these can be differentiated by various clinical and, more importantly, serologic criteria. The serologic differentiation was made possible by the discovery of an *antigen* in the blood and tissues of individuals suffering from "serum hepatitis" ("long-incubation hepatitis," MS-2); this antigen is variously referred to as Australia antigen (Aul) or hepatitis-associated antigen (HAA).<sup>295,372</sup> HAA is not found in individuals suffering from "infectious hepatitis" ("short-incubation hepatitis," MS-1).<sup>331</sup> Anti-HAA antibodies also appear in the serum of some patients with serum hepatitis,<sup>313,315</sup> especially on secondary exposure,<sup>334</sup> but never in patients with infectious hepatitis.

Unfortunately both forms of hepatitis can be transmitted by the parenteral (or oral) route and routine testing of donors for HAA will therefore not detect carriers of the infectious hepatitis virus. Nevertheless, all prospective donors must be tested for HAA, since HAA is implicated in 25 to 30% of patients with post-transfusion hepatitis<sup>313,315,374</sup> and rapid techniques for its detection are available.<sup>314,322a,353</sup>

The reported incidence of viral hepatitis following transfusion of blood and blood products varies widely and is influenced by several factors:

1. When the diagnosis is based on clinical evidence such as *icterus*, the reported overall incidence is considerably less than 1% per unit of blood administered,<sup>36,245,318</sup> but it may be in excess of 50% when HAA-positive blood is given.<sup>294</sup> However, when the diagnosis is based on elevated enzyme levels, the incidence is much higher and may reach 3%<sup>315</sup> for recipients of HAA-negative blood and 50% to nearly 100% for recipients of HAA-positive blood.<sup>315,331</sup>

2. Because of the high degree of infectivity of the hepatitis viruses, pooled blood products constitute a particular hazard, especially when materials such as coagulation factors (page 486) are prepared from exceptionally large donor pools.

3. When blood is derived from commercial sources, rather than from volunteer donors, the risks of hepatitis are particularly high. In one study the number of HAA-positive units was 13-fold greater in commercial donor blood than in volunteer blood,<sup>315</sup> and similar differences probably exist for the infectious hepatitis virus for which no specific test is yet available.

4. The incidence of hepatitis also is influenced by the care taken in screening potential donors.<sup>291</sup> Implementation of the following recommendations may aid in reducing the incidence of post-transfusion hepatitis:<sup>374</sup> (a) All prospective donors must be tested for HAA. This technique will detect 25 to 30% of hepatitis carriers. Blood containing anti-HAA antibody is apparently quite safe.<sup>291</sup> (b) It is probably well to select donors from permanent residents of the community. Do-

nors of the skid-row type, donors with tattoos, or those who are known alcoholics and drug addicts, including individuals who *habitually* use marijuana, should be avoided. When blood is obtained from outside suppliers, their standards should be ascertained. All those in close contact with hepatitis patients are excluded from donating blood for six months following contact and should be tested for HAA. Eliminating commercial donors and HAA-positive individuals may decrease the incidence of hepatitis by as much as 95%.<sup>291</sup> (c) It is important that blood banks have information about all cases of clinical hepatitis in their area. If a patient with hepatitis is the recipient of a recent transfusion, all of his donors should be investigated. Abnormal bilirubin and enzyme levels, a positive finding on HAA testing or abnormally high gammaglobulin levels in *unimplicated* donors<sup>296</sup> should lead to their exclusion from donor panels. (d) Since the risk of hepatitis is clearly related to the number of transfusions received, the judicious use of blood and blood products cannot be overemphasized.

It has been suggested that the routine addition of modified gamma globulin to blood may decrease the incidence of post-transfusion hepatitis.<sup>327</sup> Others have shown that gamma globulin from *conventional* sources only neutralizes the infectivity of serum containing the infectious hepatitis virus, but does not consistently ameliorate the infectivity of serum containing the serum hepatitis virus.<sup>331</sup> On the other hand, when gamma globulin is prepared from plasma known to contain anti-HAA antibody, very potent preparations of "SH immune globulin" can be prepared that warrant clinical trials.<sup>355</sup> Encouraging results have already been obtained in the prevention of endemic HAA-positive hepatitis by the prophylactic use of gamma globulin prepared from paid donors<sup>313</sup> known to have a high incidence of anti-HAA antibodies. Such material may also prevent serum hepatitis after accidental inoculation with material containing HAA.<sup>332</sup>

*Other infections transmitted by transfusion* include syphilis,<sup>302,359</sup> malaria,<sup>292,300,303,309</sup> toxoplasmosis,<sup>368</sup> brucellosis,<sup>353</sup> and cytomegalovirus infection.<sup>354</sup> Screening of blood

ing from the use of large amounts of old "bank blood" and hemorrhagic manifestations.

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It has been suggested that the routine use of modified gamma globulin may decrease the incidence of transfusion hepatitis.<sup>327</sup> Others have found that gamma globulin from commercial sources only neutralizes the infectious hepatitis serum containing the infectious hepatitis virus, but does not consistently ameliorate the infectivity of serum containing the hepatitis virus.<sup>331</sup> On the other hand, gamma globulin is prepared from plasma known to contain anti-HAA antibodies; potent preparations of "SH immune plasma" can be prepared that warrant further trials.<sup>355</sup> Encouraging results have been obtained in the prevention of experimental HAA-positive hepatitis by the prophylactic use of gamma globulin prepared from donors<sup>313</sup> known to have a high incidence of anti-HAA antibodies. Such material may prevent serum hepatitis after accidental contamination with material containing HAA.

Other infections transmitted by transfusion include syphilis,<sup>302,359</sup> malaria,<sup>292,399</sup> toxoplasmosis,<sup>368</sup> brucellosis,<sup>383</sup> and measles virus infection.<sup>354</sup> Screening of

by serologic tests for syphilis is not entirely adequate since in the most infectious phase of the disease (late primary, early secondary) the reactions are negative. *Treponema pallidum* is destroyed, however, if blood is kept at refrigerator temperatures for 96 hours.<sup>377</sup> Plasmodia can survive refrigeration and storage. Prophylactic treatment of donors with antimalarials is sometimes practiced in countries where malaria is endemic.

Another serious, although fortunately uncommon, hazard is from the administration of contaminated blood. This is due to the accidental introduction of saprophytic bacteria into the bottle of blood. Gram-positive saprophytes, such as diphtheroids, generally produce only fever. However, gram-negative bacteria have been responsible for the production of profound shock that is almost always fatal.<sup>378</sup> Many gram-negative bacteria appear to be capable of utilizing citrate as the sole source of carbon and can multiply in the refrigerator at 4 to 8° C. *Pseudomonas* is one of the most common offenders. The coli-acrogenes variety exhibits good growth in the incubator and at room temperature but not always in the refrigerator. After a latent period of 30 minutes or more, fever, hypotension, and pain in the abdomen and extremities develop and death from shock may take place within six hours. Severe vomiting and diarrhea occur and, if the patient survives for 24 hours, signs of renal failure develop. A hemorrhagic diathesis may ensue. Differentiation from a reaction due to the administration of incompatible blood can be made by microscopic examination of a gram stain of blood remaining in the bottle and by the absence of evidence of a hemolytic reaction. Antibiotics are of limited or no value as prophylactic additives to the blood.

### Miscellaneous III Effects

*Thrombophlebitis* is not rare when transfusions are given repeatedly and especially if polyethylene catheters or small veins are used, particularly in the lower extremities.

*Hemosiderosis* closely resembling hemo-

chromatosis may result from the administration of a large number of transfusions.

*Fat embolism* may occur if positive pressure is used when blood is being given via the bone marrow or in patients who have bony injuries. Secondary osteomyelitis or mediastinitis and cardiac tamponade can follow such transfusions. *Air embolism*, resulting from the accidental introduction of air as, for example, through ill-fitting tubing or when blood is introduced under pressure, is usually well tolerated if the amount is small (<30 ml) but may be dangerous in a gravely ill patient.

## Clinical Use of Plasma and Plasma Derivatives

The large-scale use of plasma began during World War II when plasma was introduced as a volume expander in the treatment of shock under field conditions. Plasma is still given as a volume expander, but now its value as a source of special fractions, such as clotting factors, albumin, and gamma globulins, far outstrips all other uses. The role of plasma derivatives in the therapy of disease will be discussed elsewhere in the appropriate clinical context; this section is intended as a general introduction to the clinical use of plasma and plasma derivatives.

### Plasma

**COLLECTION.** Plasma is generally prepared from single-donor units of fresh blood after the cellular elements have been removed for use in transfusion. Other sources include outdated blood and plasmapheresis of volunteer donors, usually for the preparation of special plasma derivatives.<sup>413</sup> In plasmapheresis<sup>401</sup> a unit of blood is removed at a time from the donor and centrifuged. The supernatant plasma is separated and the packed cells are reinfused in the donor immediately. In this way two units are readily processed at one time and the same procedure may be repeated twice weekly without changing the plasma protein patterns of the donor to any extent.<sup>414</sup> Even larger volumes



of plasma may be obtained by continuous flow centrifugation techniques.<sup>387,402a</sup>

**STORAGE.** Since plasma is usually a by-product of blood transfusion, it contains ACD or CPD anticoagulant (page 473). EDTA or citrate formulas without dextrose may be used if blood is collected specifically for plasma fractionation. Heparin is not a suitable anticoagulant if the plasma is to be stored for more than two days.

Storage conditions depend on the projected use of the product. When plasma is to be used as a volume expander or as a source of albumin it may be stored at 15 to 30° C up to three years.<sup>421</sup> Albumin is stable under these conditions, although most coagulation factors deteriorate rapidly. In addition, fibrin degradation products (Chapter 38) that induce hemostatic defects following transfusion may develop.<sup>398</sup> If plasma is to be used for replacement therapy in disorders of coagulation, it must be frozen or freeze-dried within a few hours of collection.<sup>421</sup>

**INDICATIONS.** In the past, plasma has been used mainly as a volume expander, but for patients in acute traumatic shock whole blood is usually the therapy of choice (page 475); Ringer's lactate<sup>406</sup> or other plasma expanders such as dextran, plasma protein fraction (PPF), or albumin (see below) may be equally effective in an emergency. Plasma, PPF, or albumin is also indicated in the therapy of burns.

Fresh-frozen or freeze-dried plasma is a useful source of coagulation factors including factors V and VIII. Other factors such as I, II, VII, IX, X, XI, XII, and XIII may be obtained in somewhat reduced quantities from outdated blood stored at 4° C, although fresh-frozen plasma is usually preferable (see Chapter 37 for details).

Plasma also has been used in the therapy of hypogammaglobulinemia (Chapter 44) and as a source of CI-esterase inhibitor in the treatment of hereditary angioneurotic edema.<sup>388</sup>

**COMPLICATIONS.** The complications of plasma therapy are similar to those of whole

blood transfusion. They include (1) allergic reactions to white cell and platelet antigens or gamma globulin components<sup>304,364,391</sup>; (2) citrate toxicity (page 486); (3) bacterial contamination; and (4) hepatitis. The last-named probably is the most serious complication and adequate precautions (page 487) need to be taken. Pooled plasma should never be used. Whenever possible, plasma fractions should be given, rather than whole plasma since the hepatitis-associated antigen is probably removed in their preparation.<sup>56</sup>

### Plasma Fractions

The traditional cold ethanol method developed by Cohn during World War II is still the basis for plasma fractionation in the United States.<sup>389,409,413</sup> The procedure is based on the fractional precipitation of plasma proteins with ethanol at low temperatures and low salt concentrations. Proper sequential adjustment of the concentration of protein salts and ethanol as well as of temperature and pH permits the isolation of six fractions, each of which can be subfractionated by appropriate procedures into relatively pure components. Other fractionation schemes utilize ether,<sup>410</sup> polyphosphates,<sup>408</sup> combinations of ammonium sulfate and Rivanol,<sup>396</sup> and polyethylene glycol or glycine.<sup>410</sup> Still other methods employ specific adsorbants such as cellulose derivatives.<sup>416,417</sup> Immunoabsorbants are under investigation for the preparation of purified antibodies to diphtheria, tetanus, and other bacterial infections.<sup>413</sup>

### Albumin Preparations

Solutions rich in albumin can be produced by a variety of techniques,<sup>389,408</sup> but that produced by alcohol fractionation tends to be the purest (>95% albumin). The material may be heated to 60° C for 10 hours to prevent the transmission of hepatitis.<sup>392</sup> In a less elaborate variation of this method a preparation containing 85 to 90% albumin may be prepared (plasma protein fraction, PPF); this is as useful clinically as the more expensive

product of the original Cohn method.<sup>397, 403, 419</sup> It too can be heated to destroy the hepatitis virus.

Albumin has been used in the treatment of patients in shock due to hemorrhage, trauma, or burns,<sup>391, 399</sup> and for the therapy of patients with hypoalbuminemia due to cirrhosis or chronic renal disease. In the latter group, it may be of little permanent value, since it is often excreted as quickly as it is infused.

Albumin and PPF are marketed as 5 g/dl solutions.<sup>420</sup> This concentration is most frequently used as a plasma expander. Twenty-five grams of albumin are the osmotic equivalent of about 500 ml (5 dl) of plasma. Albumin also comes in a 25 g/dl solution that is low in sodium and is particularly useful in the treatment of patients with protein-deficiency states and burns. It should not be given to dehydrated patients without the concomitant infusion of other fluids.

### *Immunoglobulins*

Immunoglobulins may be prepared by cold ethanol fractionation,<sup>489</sup> by ether fractionation,<sup>407</sup> or by precipitation with ammonium sulfate. The cold ethanol method yields about 95% IgG with little IgA or IgM.<sup>396</sup> The preparation is marketed as a 16 g/dl solution,<sup>420</sup> representing a 16-fold concentration of plasma IgG. It should always be given intramuscularly since adverse reactions are encountered when it is given by the intravenous route.<sup>386</sup> These reactions are thought to be due to aggregates and can be prevented by the use of immunoglobulin preparations treated with proteolytic enzymes<sup>386, 412</sup> or incubated at a low pH.<sup>386</sup> The biologic half-life of such preparations is decreased, however.<sup>402, 405</sup>

Specific immunoglobulins against a number of infectious agents are available and others are being developed.<sup>411</sup> Anti-Rh<sub>0</sub> globulin is discussed in Chapter 27.

Various investigations have shown that IgA and IgM globulins differ from IgG in regard

to the antibodies that they contain.<sup>404, 418</sup> Immunoglobulin preparations containing from 15 to 20% each of IgA and IgM globulins are now available.<sup>411</sup>

The use of immunoglobulins in patients with immune deficiency syndromes is discussed in Chapter 44. Generally 100 to 300 mg/kg are given prophylactically every three to four weeks, so as to assure a minimum circulating IgG level of no less than 200 mg/dl. *Prophylactic therapy* is particularly useful in patients with sex-linked congenital agammaglobulinemia, transient hypogammaglobulinemia of childhood, late-onset agammaglobulinemia, and agammaglobulinemia with thymoma. Surface infections involving the respiratory tract and the intestinal tract are not prevented by this therapy. During *acute infections* of other kinds it is well to give additional injections of 200 to 300 mg/kg.

The role of gamma globulin in the prevention of hepatitis has been discussed previously (page 487).

### *Clotting Factors*

The preparation of clotting factors and their use in the therapy of coagulation disorders are discussed in Chapters 37 and 38.

### *Plasma Enzymes and Enzyme Inhibitors*

At least four plasma enzymes and enzyme inhibitors have been isolated, although their clinical application is still in the investigational stage.<sup>411</sup> Plasminogen has been tried in the therapy of patients with hyaline membrane disease<sup>400</sup>; serum cholinesterase may be useful in the therapy of persons who have taken overdoses of succinylcholine<sup>390</sup> and of those poisoned by alkyl phosphates.<sup>393</sup> Cl-esterase inhibitor may be lifesaving in patients suffering from hereditary angioneurotic edema.<sup>411</sup> Antithrombin III (Chapter 38) is being tried in the therapy of intravascular coagulation.<sup>411</sup>

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# *Leukocyte and Platelet Antigens and Transfusions*

- Leukocyte Antigens
  - History
  - HL-A System
  - Non-HL-A Leukocyte Antigens
  - Clinical Significance of Leukocyte Antigens
- Leukocyte Transfusions
  - Indications
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  - Methods of Detection
  - Nature of Antiplatelet Antibodies
  - Clinical Significance of Platelet Antigens
- Platelet Transfusion
  - Collection and Preservation
  - Matching Procedures
  - Administration
  - General Indications
  - Complications

but the transfusion of granulocytes and lymphocytes must still be regarded as experimental and is for the most part restricted to special centers.

The transfusion of leukocytes and platelets is complicated by the existence of antigens other than the blood group determinants discussed in the previous chapter. Exposure to foreign antigens of this type evokes the production of powerful antibodies that may render useless the transfusion of platelets or leukocytes carrying the corresponding determinants.

## **Leukocyte Antigens**

### **History**

Research on leukocyte antigens and organ transplantation has been inextricably intertwined and rightly so, because, with rare exceptions, most presently recognized leukocyte antigens are also found on other tissues, where they act as major determinants of the success or failure of tissue transplants. Thus the terms "leukocyte antigens," "transplantation antigens," and "histocompatibility antigens" are used interchangeably. The first proof that leukocytes carry transplantation antigens was provided by Medawar, who, in 1946, showed that graft rejection is an immunologic phenomenon and that a state of homograft sensitivity can be induced by an

THE need for effective transfusion of platelets and leukocytes has long been recognized, but the techniques for collecting and transfusing these blood components in a viable state have developed much more slowly than those for red cells. Platelet transfusions are now available in most hospitals,

intradermal injection of leukocytes.<sup>69</sup> Similar observations have since been made in many species including man.<sup>37,91</sup> Conversely, it was also shown that antileukocyte antibodies appear after tissue transplantation in animals and man,<sup>3,135</sup> again suggesting that histocompatibility testing is almost synonymous with leukocyte grouping.

The systematic study of leukocyte antigens in man began in 1954; Dausset, in a study of 60 persons with leukoagglutinating antibodies, found that 90% of these individuals had received multiple transfusions.<sup>23</sup> Other studies confirmed the role of transfusion in the induction of antileukocyte isoantibodies<sup>71,89</sup> and showed that isoantibodies against leukocytes may also appear during pregnancy,<sup>87,101</sup> the latter being formed against fetal leukocyte antigens inherited from the father.

In 1958, Dausset observed that six individuals who had received multiple small transfusions from a single donor all showed a similar pattern of reactivity against a panel of foreign leukocytes.<sup>24</sup> He concluded that these individuals lacked the same antigen, which he named Mac. About 60% of the French population possess this antigen, now referred to as HL-A2. An antigen identical to Mac and three other antigens were described by Killman in 1958<sup>53</sup> and in 1959 Van Rood and associates identified two new antigens, 2 and 3, by the use of leukocyte agglutinins formed after pregnancy.<sup>102</sup>

Van Rood and Eernisse,<sup>97</sup> in describing the first leukocyte group system, showed that the antigens 4a and 4b (leukocyte Group Four) behaved as if they were determined by alleles. Family studies subsequently confirmed this conclusion and indicated that the 4a and 4b genes are inherited as simple codominant mendelian characters.<sup>99</sup> Subsequently, many other leukocyte groups were described and designated by a confusing array of letters and numerals: Group Five (5a and 5b), Group Six (6a and 6b), Group Seven (7a, 7b, 7c, 7d), Eight (8a) and Nine (9a) by van Rood and coworkers<sup>100,103</sup>; PI<sup>a</sup>GrLy<sup>b1</sup> and PI<sup>a</sup>GrLy<sup>c1</sup> by Shulman and associates<sup>114</sup>; LA1 and LA2 by Payne and colleagues<sup>88</sup>; and LA3 by

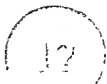
Bodmer and coworkers<sup>15</sup>; the Hu-1 system by Dausset.<sup>24</sup>

Histocompatibility workshops in 1965 (Leyden)<sup>45</sup> and in 1967 (Torino)<sup>46</sup> clearly established that most of the leukocyte antigens defined by the sera of 16 participating laboratories were determined by closely linked genes at one chromosomal region, which was called the HL-A locus<sup>19,46,105</sup> (Human Leukocyte, locus A<sup>54</sup>). At the same time a new system of designations was recommended by which a well-defined factor would be indicated by a number following the symbol for the system, such as HL-A1, HL-A2, HL-A6, etc.<sup>80</sup> When a factor is confirmed by several laboratories but does not yet qualify for an HL-A designation, it is given a provisional number preceded by the prefix W, eg W21.<sup>141</sup> Specificities discovered by individual laboratories carry designations identifying the laboratory of origin, eg Te6 (Terasaki, Table 12-1). Such specificities may eventually be elevated to W or HL-A status. The current series of recognized HL-A antigens and the corresponding older designations used in various laboratories are given in Table 12-1.

## The HL-A System

### Genetics

The major antigens found on human leukocytes are determined by a single chromosomal segment, the "major histocompatibility complex" (MHC) which is situated on an as yet unidentified pair of autosomes. Those portions of the MHC which determine the major serologically defined histocompatibility antigens of man have been named the HL-A loci.<sup>80</sup> Two closely linked loci are presently well defined and are referred to as the LA locus and the Four locus. Additional loci may exist,<sup>54</sup> but this has been denied by some researchers. Each of the two major loci is characterized by an as yet unknown number of genes that behave as multiple alleles, since only one LA allele and one Four allele is present on each of a pair of chromosomes. The antigens presently known to be products



## *Leukocyte and Platelet Antigens and Transfusions*

### Leukocyte Antigens

#### History

#### HL-A System

#### Non-HL-A Leukocyte Antigens

#### Clinical Significance of Leukocyte Antigens

### Leukocyte Transfusions

#### Indications

#### Selection of Donors

#### Collection of Granulocytes

#### Transfusion of Granulocytes

#### Complications

### Platelet Antigens

#### HL-A Antigens

#### Other Isoantigens Shared by Platelets and Leukocytes

#### ABO Antigens

#### Platelet Specific Isoantigens

#### Methods of Detection

#### Nature of Antiplatelet Antibodies

#### Clinical Significance of Platelet Antigens

### Platelet Transfusion

#### Collection and Preservation

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#### Administration

#### General Indications

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THE need for effective transfusion of platelets and leukocytes has long been recognized, but the techniques for collecting and transfusing these blood components in a viable state have developed much more slowly than those for red cells. Platelet transfusions are now available in most hospitals,

intra-dermal injection of leukocytes.<sup>69</sup> Similar observations have since been made in many species including man.<sup>37,91</sup> Conversely, it was also shown that antileukocyte antibodies appear after tissue transplantation in animals and man,<sup>3,123</sup> again suggesting that histocompatibility testing is almost synonymous with leukocyte grouping.

The systematic study of leukocyte antigens in man began in 1954; Dausset, in a study of 60 persons with leucoagglutinating antibodies, found that 90% of these individuals had received multiple transfusions.<sup>23</sup> Other studies confirmed the role of transfusion in the induction of antileukocyte isoantibodies<sup>71,86</sup> and showed that isoantibodies against leukocytes may also appear during pregnancy,<sup>87,101</sup> the latter being formed against fetal leukocyte antigens inherited from the father.

In 1958, Dausset observed that six individuals who had received multiple small transfusions from a single donor all showed a similar pattern of reactivity against a panel of foreign leukocytes.<sup>21</sup> He concluded that these individuals lacked the same antigen, which he named Mac. About 60% of the French population possess this antigen, now referred to as HL-A2. An antigen identical to Mac and three other antigens were described by Killion in 1958<sup>53</sup> and in 1959 Van Rood and associates identified two new antigens, 2 and 3, by the use of leukocyte agglutinins formed after pregnancy.<sup>102</sup>

Van Rood and Eernisse,<sup>97</sup> in describing the first leukocyte group system, showed that the antigens 4a and 4b (leukocyte Group Four) behaved as if they were determined by alleles. Family studies subsequently confirmed this conclusion and indicated that the 4a and 4b genes are inherited as simple codominant mendelian characters.<sup>99</sup> Subsequently, many other leukocyte groups were described and designated by a confusing array of letters and numerals: Group Five (5a and 5b), Group Six (6a and 6b), Group Seven (7a, 7b, 7c, 7d), Eight (8a) and Nine (9a) by van Rood and coworkers<sup>100,103</sup>; P1GrLy<sup>B1</sup> and P1GrLy<sup>G1</sup> by Shulman and associates<sup>114</sup>; LA1 and LA2 by Payne and colleagues<sup>88</sup>; and LA3 by

Bodmer and coworkers<sup>15</sup>; the Hu-1 system by Dausset.<sup>24</sup>

Histocompatibility workshops in 1965 (Leyden)<sup>15</sup> and in 1967 (Torino)<sup>16</sup> clearly established that most of the leukocyte antigens defined by the sera of 16 participating laboratories were determined by closely linked genes at one chromosomal region, which was called the HL-A locus<sup>19,16,105</sup> (Human Leukocyte, locus A<sup>54</sup>). At the same time a new system of designations was recommended by which a well-defined factor would be indicated by a number following the symbol for the system, such as HL-A1, HL-A2, HL-A6, etc.<sup>60</sup> When a factor is confirmed by several laboratories but does not yet qualify for an HL-A designation, it is given a provisional number preceded by the prefix W, eg W21.<sup>141</sup> Specificities discovered by individual laboratories carry designations identifying the laboratory of origin, eg Te6 (Terasaki, Table 12-1). Such specificities may eventually be elevated to W or HL-A status. The current series of recognized HL-A antigens and the corresponding older designations used in various laboratories are given in Table 12-1.

## The HL-A System

### Genetics

The major antigens found on human leukocytes are determined by a single chromosomal segment, the "major histocompatibility complex" (MHC) which is situated on an as yet unidentified pair of autosomes. Those portions of the MHC which determine the major serologically defined histocompatibility antigens of man have been named the HL-A loci.<sup>60</sup> Two closely linked loci are presently well defined and are referred to as the LA locus and the Four locus. Additional loci may exist,<sup>54</sup> but this has been denied by some researchers. Each of the two major loci is characterized by an as yet unknown number of genes that behave as multiple alleles, since only one LA allele and one Four allele is present on each of a pair of chromosomes. The antigens presently known to be products

**Table 12-1. Leukocyte Antigens: HL-A Nomenclature and Previously Used Equivalents**

[illegible]

## SECOND (FOUR) SERIES

## Equivalent Nomenclature

HL-A Official or Workshop Designation	Amos	Batchelor	Bodmer	Cepellini	Dausset & Colombani	Engelfriet	Jeannef	Kissmeyer	Kayr	Morris	Morton & Pickles	Payne	Terasaki	Thorsby	van Rood	Walford
HL-A 5 (subdivision)	Ao 12	Bi 25	4c	To 5	Da 5	CLB 7		HLA 5-AJ HLA 5*				4c	Ta 11 (old)	MH HLA 5-AJ HLA 5*	(1)	<Lc-19
HL-A 7	Ao 2	<Bi 4	4d	To 7	Da 10	CLB 1	<GE 4	T 12		Sly 1	Bga	4d	Ta 9	TT	<7c = 6c, 6b 7d	Lc 8
HL-A 8	Ao 15	>Bi 6	4a11	To 11	Da 4	CLB 14		HN					Ta 26	HN	(1)	Lc-7
HL-A 12	HK	Bi 23	<4c*	To 21	Da 20	CLB 22	GE 11	R*					Ta 5 or 5D		<6b (1)	Merrit A
HL-A 13	Ao 13			To 23			GE 23	B8					Ta 10 or 8D		(1)	>Lc-16
W 5								JA						KSO*	<6b	
W 10 (subdivision)					Da 18	CLB 10	<GE 17	LND	MaKi				Ta 14 or 54	LND	<7a (1)	Lc-25
W 14		>Bi 26			Da 23			LND AJ					Ta 15 or 55	LND AJ		
W 15 (subdivision)							>GE 12	U 18					Ta 64	LND*	(1)	
W 16 (subdivision)	Ao 81				Da 31		GE 15	SL MaPh	MaPh	<M 2	Bgb		Ta 17 or 57	SL MaPh	Orina (1)	
W 17	Ao 70	Bi 12					GE 28	CM					Ta 18 or 58	CM	(1)	
W 18 (subdivision)		Bi 20	<4c*					SLCM						SLCM		
W 18 (subdivision)								CM*		<M 3			Ta 61	CM*	7ba (1)	
W 21 (subdivision)					Da 24									ET		
W 21 (subdivision)														SL ET		
W 21 (subdivision)														ET*		
W 22 (22 1)		Bi 22		To 28			GE 22	AA					Ta 22 or 51	AA	<7c	
W 22 (22 2)					Da 30			AA*						AA*		
W 27 (subdivision)	<Ao 10				Da 34	HR	<GE 18	AA AJ					Ta 27 or 52	AA AJ	<7c	
								FJH						FJH AJ		
								FJH AJ						FJH*		
								FJH*								
W 4	Ao 27			<To 2	Da 3										4a	Lc-13
W 6	Ao 72			To 6	Da 7										4b	Lc-14
															7b	Lc-15
Without equivalents	Ao 78				Da 34	HR		407*						TT*	6a	Lc-21
	Ao 56							TT*								Lc-27
	Ao 79															
	Ao 18															

From WHO Terminology Report,<sup>111</sup> courtesy of Histocompatibility Testing 1972

Note The specificities listed under HL-A designations eg W 23 and W 24 under HL-A 9 indicate subfactors which are part of the broader HL-A specificity

of these two segregant series of genes are listed in Table 12-1. The First or LA series contains HL-A 1,2,3,9,10, 11, and some antigens not yet designated by the HL-A nomenclature. The Second "Four" series contains the HL-A 5,7,8,12, and 13 antigens as well as others. The genetic determinants within each series behave as mutually exclusive genes or alleles. Thus a given person may have a maximum of two first series and two Second series antigens, one First series and one Second series antigen being determined by each chromosome. The antigens determined by the same chromosome are referred to as *haplotypes*.

The LA and Four loci appear to be situated very close to each other within the MHC, since very few recombinations have been described.<sup>54</sup> Because of this close linkage the antigens defined by a single chromosome are inherited as units or "packets." Thus only four types of children can result from any given marriage (Fig. 12-1). No parent has yet been found to hand down two antigens belonging to the same series to his

children. If, for instance, one of the parents possesses antigens HL-A1 and HL-A2 (both from the First series) and the other one does not, the children always receive either HL-A1 or HL-A2, but never both.

The gene frequencies for several U.S. population groups are given in Table 12-2. If there were no association between individual First and Second series determinants, the frequency of each haplotype would, of course, depend only on the gene frequencies of the individual alleles. It has been shown, however, that some haplotypes occur much more frequently than others. Among Caucasians the strongest association is HL-A1, 8 and HL-A3, 7, but others have also been described.<sup>49,121</sup> The cause of this phenomenon is unknown.

Racial differences in HL-A antigens have been noted and may in the future serve as useful anthropologic markers.<sup>48</sup> Thus HL-A1 may be a unique characteristic of Caucasian populations,<sup>15</sup> since it appears to be absent among Japanese,<sup>114</sup> Australian aborigines,<sup>54</sup> pygmies,<sup>15</sup> and American Indians,<sup>14</sup> and is

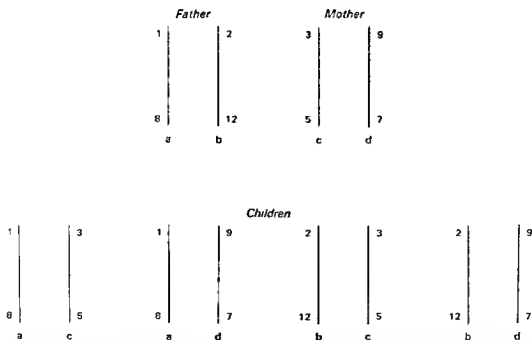


Fig. 12-1 The inheritance of HL-A antigens. The letters a b and c d designate paternal and maternal chromosomes respectively and the numbers refer to HLA antigens. The specificities determined by any given chromosome are referred to as haplotypes and are inherited as units.



Table 12-2. HL-A Gene Frequencies in Three Population Groups

	First Locus				Second Locus		
	Caucasian	Oriental	Black		Caucasian	Oriental	Black
HL-A1	0.125	0.00	0.09	HL-A5*	0.07	0.18	0.06
HL-A2	0.265	0.25	0.135	HL-A7	0.10	0.03	0.085
HL-A3	0.145	0.01	0.09	HL-A8	0.09	0.00	0.035
HL-A9	0.105	0.375	0.14	HL-A12	0.145	0.08	0.10
HL-A10	0.055	0.08	0.025	HL-A13	0.02	?	?
HL-A11	0.06	0.09	0.02				

\*Te 11

After Albert et al. *Histocompatibility Testing* 1970<sup>47</sup>

present in a much lower frequency in American Negroes and Maoris than in Caucasians.<sup>15</sup> In addition, the observed associations of HL-A1, HL-A8 and HL-A3, HL-A7 among Caucasians do not show up in other populations.

HL-A8 is of low frequency in Japanese; HL-A10 is of higher frequency among Orientals and Negroes than among Caucasians<sup>118</sup>; and Te10 is common among Orientals. Pygmies also have a much higher incidence of "blank" alleles in both series of antigens than do Caucasians.<sup>15</sup>

In Greenland Eskimos there is a very high incidence of HL-A9 (81%), FJH (38%), and HL-A5. The latter is four times as common among Eskimos as among Northern Europeans, but HL-A1, HL-A8, Da 17, LI, HL-A7, HL-A10, and SL are of very low frequency.<sup>54</sup>

In addition to the two HL-A loci, the major histocompatibility complex contains regions that determine other aspects of lymphocyte structure and function. One of these is the MLC (mixed leukocyte culture) or MLR (mixed leukocyte reaction) locus,<sup>83,27,142</sup> which is closely linked to the HL-A "Four" region of the chromosome and whose products are responsible for the strong stimulation of lymphocytes in mixed leukocyte cultures (Chapter 7 and below) *in vitro*, and the graft-versus-host (GvH) response (Chapter 7) *in vivo*.<sup>83,27,73a,142</sup> In mice the GvH reaction as well as the MLC reaction is directed against the products of immune response (Ir) genes, which are also closely linked to the H-2K locus (comparable to the

Four locus in man) and which control the immune response to a large number of antigens.<sup>13a,55</sup> HL-A associated Ir genes may also occur in man,<sup>18</sup> but their relation to the MLC locus is not yet established. The lymphocyte-stimulating antigens appear to be present in T cells (Chapter 7) only, since lymphocytes from patients with thymic dysplasia (Nezeloff's syndrome, Chapter 44), though carrying HL-A antigens, do not stimulate lymphocytes of HL-A nonidentical individuals.<sup>27</sup> Experiments in rodents have also confirmed the thymus dependence of stimulating lymphocytes<sup>27</sup> and the expression of immune response (Ir) genes in thymus derived cells.<sup>13a</sup>

### Methods of Detection

Many methods have been introduced for the detection of leukocyte antigens<sup>54</sup> including: (1) leucoagglutination techniques; (2) cytotoxicity tests, which depend on the complement-dependent disruption of the cell membrane and the demonstration of cell death by phase contrast microscopy, penetration of the cell membrane by supravital dyes, fluorochromasia, and <sup>51</sup>chromium release; (3) complement-fixation tests. Reviews of all of these techniques and detailed notes of methodology have been published.<sup>54,79</sup> All of these tests are macrotechniques that require relatively large quantities of cells and sera, measured in millions of cells and drops of sera. Reliable and simple microcytotoxicity tests are less demanding of resources and personnel.<sup>72,123</sup> These tests can be performed with

1  $\mu$ l (or less) of antiserum and 2,000 to 3,000 cells; following an appropriate incubation period, 5  $\mu$ l of rabbit complement are added and, after further incubation, cytotoxicity is detected by one of several means, usually the uptake of a supravital dye such as trypan blue or eosin Y<sup>54</sup> (Fig. 12-2). Since platelets also contain HL-A antigens (see below), they may be used instead of lymphocytes in HL-A typing. Details of this technique are discussed on page 514.

Theoretically, typing sera could be derived from polytransfused patients, from individuals who have received organ transplants, and from animals such as chimpanzees that have been deliberately immunized with human transplantation antigens.<sup>51</sup> The most satisfactory sources of typing sera, however, are pregnant women and human volunteers who have been deliberately sensitized with foreign skin grafts and/or leukocytes.<sup>54</sup> The antibodies are frequently of rather limited specificity, since sensitization, either natural or planned, usually involves a restricted number of leukocyte antigens, eg, the antigens that a fetus has inherited from the father and that differ from those of the mother.

*Cytotoxic antibodies* may be either IgG or IgM,<sup>54 87 119,136</sup> although IgG antibodies appear to be the most common. Monospecificity of typing sera is highly desirable and could be established, in an operational sense at least, by population and family studies<sup>54</sup> as well as by absorption techniques.<sup>137</sup> However, since many sera contain cross-reacting antibodies that may be removed by absorption with either of two antigens,<sup>54</sup> absorption studies by themselves are not sufficient proof of monospecificity. Cross reactivity appears to be confined to antigens within a series,<sup>54</sup> suggesting that greater structural differences exist between antigens belonging to the two series than between antigens within a series.

#### **Correlation between HL-A Typing and Mixed Leukocyte Cultures (MLC)**

When lymphocytes from unrelated individuals are mixed and allowed to grow *in vitro*, enlargement, DNA synthesis, and cell

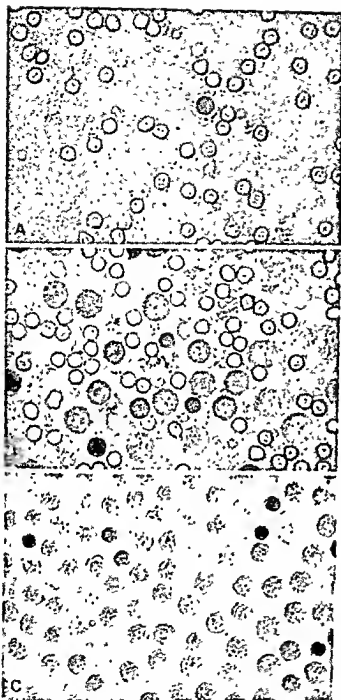
division take place, apparently reflecting a primary immune response stimulated *in vitro*. In this system, lymphocytes function not only as mediators of immunity but also as carriers of antigens. It appears that differences at major histocompatibility loci usually correlate well with the immunologic reactivity in mixed leukocyte cultures,<sup>1-4</sup> although mixed lymphocyte reactivity is now known to be governed by a locus that lies on the same chromosome as the *major* (Four and LA) loci, but is separate from them (see above).<sup>8a,27,35</sup> Generally speaking, clinical trials have shown good correlation between the results of lymphocyte typing, mixed leukocyte cultures, and the survival of allografts.<sup>8,21</sup>

The MLC test also lends itself to some quantitative evaluation of HL-A differences. This is most clearly seen in sibling studies in which individuals with identical haplotypes show no stimulation; those differing by two haplotypes exhibit maximum stimulation and those differing by a single haplotype show an intermediate degree of stimulation.<sup>1,112</sup>

Descriptions of standard methods for mixed leukocyte cultures have been published.<sup>8,9</sup> Most MLCs are now done by the one-way test in which one population of cells is pretreated with mitomycin. This population then serves as the antigen, whereas the other population furnishes the proliferating cells.<sup>9</sup> A speeded-up version of the test has also been described.<sup>11</sup>

#### **Chemical Characterization of HL-A Antigens<sup>37,94</sup>**

Unlike ABH and Lewis blood group substances, HL-A antigens are not found in secretions and the extraction of antigen from cell membranes is therefore a necessary first step in their characterization. Several methods have been described<sup>26,52 68</sup> and separation of series 1 and 2 antigens has been achieved.<sup>66</sup> Highly purified HL-A antigens have a sedimentation coefficient  $S_{20}W$  of 2.3 and a molecular weight of 31,000 daltons.



**Fig 12-2** The microcytotoxicity test. A, A negative reaction with living lymphocytes (only one stained lymphocyte seen). B, A weakly positive reaction with killing of half the cells. C, A strongly positive reaction with killing and staining of all lymphocytes. (From Kissmeyer-Nielsen and Thorsby,<sup>24</sup> courtesy of the authors and Transplantation Reviews.)

HL-A antigens are essentially polypeptide in nature<sup>78,94</sup>; antigenic activity is irreversibly destroyed by proteolytic enzymes, a variety of protein denaturants, temperatures in excess of 50°C, pH values above 10 and below 4, and detergents in concentrations sufficient to irreversibly affect protein conformation. In addition, distinct, reproducible,

and statistically significant differences in amino acid composition have been demonstrated in HL-A and H-2 antigens of different phenotypes.<sup>78-94</sup> Carbohydrates make up less than 1% of highly purified HL-A antigens. It is unlikely that carbohydrates are essential for antigenic activity *in vivo*, but this cannot be ruled out with . . .

## Distribution of HL-A Antigens

HL-A antigens have been found on both lymphocytes and granulocytes,<sup>11,116</sup> but there appear to be fewer on granulocytes than on lymphocytes.<sup>130</sup> HL-A antigens are also found on leukemic leukocytes.<sup>131</sup> No HL-A antigens have been detected on mature red cells so far,<sup>109</sup> although the Bg<sup>a</sup> antigen, which is related to HL-A7, may be an exception.<sup>74</sup> Reticulocytes appear to possess HL-A antigens<sup>44</sup> that presumably are lost or "switched off" during maturation of the cell. Mature red cells do, however, contain other transplantation antigens, notably those belonging to the ABO system. HL-A antigens are well represented on platelets (page 511).<sup>120</sup>

HL-A antigens also appear to be constituents of most normal human tissues. Those tested include skin,<sup>70</sup> kidney,<sup>70</sup> liver,<sup>106</sup> lung,<sup>106</sup> placenta,<sup>106</sup> and spermatozoa<sup>33</sup> in which a probable haploid expression has been demonstrated. HL-A antigens are also detectable on various tissue culture lines.<sup>93</sup>

## Non-HL-A Leukocyte Antigens

Non-HL-A leukocyte antigens found on leukocytes include determinants of the ABO system<sup>34</sup> as well as the antigens NA1, NB1, and Vaz, 5a/5b and 9a. The ABH determinants have been discussed in Chapter 11. The NA1,<sup>58</sup> NB1,<sup>57</sup> and Vaz<sup>59</sup> antigens are of particular interest since they appear to be granulocyte specific. They segregate independently of the HL-A antigens. Antibodies against the latter antigens may lead to neonatal granulocytopenia<sup>57,58,59</sup> (Chapter 42).

The diallelic antigens 5a and 5b<sup>62</sup> have a gene frequency of .20 and .80, respectively. Anti-5b sera are quite common, but anti-5a sera are rare<sup>61</sup>; both sera give agglutination reactions only.<sup>61</sup> The 5a/5b antigens do not share the HL-A locus and do not appear to function as transplantation antigens.<sup>124</sup>

The 9A antigen, also described by van Rood, apparently is not associated with any of the other antigens.<sup>124</sup>

## Clinical Significance of Leukocyte Antigens

### Transplantation

A large body of evidence now indicates that the survival and function of tissue transplants are noticeably affected by the degree of donor-recipient compatibility within the HL-A system<sup>34</sup> especially at the Four locus.<sup>107</sup> For while the activation of lymphocytes in MLC is dependent on incompatibilities determined by non-HL-A loci (above), the destruction of target cells in sensitized individuals appears to be mediated by cells and/or antibodies with specificity for HL-A antigens.<sup>27,73a</sup> At the same time it is obvious that other tissue antigens also function as transplantation antigens, notably those belonging to the ABO system (Chapter 11), and, probably, a large number of "minor" histocompatibility antigens whose identity is not yet established, but whose combined importance may rank with that of any one HL-A antigen.

The survival time of *skin transplants* is definitely affected by the compatibility of HL-A antigens,<sup>3,54,104,112,138</sup> although in most studies this correlation is only noted when transplants are exchanged between related pairs.<sup>20,104,138</sup> Nevertheless, leukocyte typing appears to be useful when skin grafts are selected for patients suffering from severe burns; skin from closely matched unrelated donors shows much better survival than that from poorly matched donors.<sup>13</sup> However, even in HL-A and ABO identical siblings, skin transplants seldom survive more than twice as long as those from incompatible donors, which clearly indicates that antigens other than those belonging to the HL-A and ABO systems also are important in skin transplantation. The degree of stimulation in mixed leukocyte cultures appears to be a good prognosticator of the fate of skin transplants in nonsensitized individuals, but is probably of little value in sensitized pa-

tients.<sup>107</sup> Others have found no correlation between MLC responses and skin-graft survival.<sup>112</sup>

The survival and function of *kidney transplants* also depend on the goodness of the histocompatibility match.<sup>51,131</sup> Results are universally satisfactory when HL-A identical siblings are used as donors,<sup>54,56</sup> and good results also are obtained when the recipient has one or two antigens not present in the donor. The course of mismatched transplants, when the donor has one or two antigens not present in the recipient, is much less satisfactory, irrespective of whether the donor is a sibling, parent, or unrelated individual.<sup>54,56</sup> The survival of kidneys from closely matched unrelated donors (cadaver transplants) is also better than that from poorly matched donors.<sup>82</sup> The presence of preformed antileukocyte antibodies in the recipient indicates a poor prognosis for kidney survival, even when donors and recipients are well matched.<sup>83</sup> The data for *heart transplants* are sparse, but suggest that similar relationships between prognosis and the goodness of the match exist.<sup>54</sup>

*Bone marrow transplants* (Chapter 44) present special problems since they involve the transfer of immunologically competent cells into an immunologically crippled host. When histocompatibility differences exist, graft-versus-host (GvH) disease (Chapter 7) complicates the course of almost all patients and the host-versus-graft response (homo-graft rejection, Chapter 7) often results in transplant failure. GvH disease is characterized by dermatitis, hepatitis, hemolytic anemia, and thrombocytopenia, and eventually by a severe wasting syndrome, bone marrow aplasia, lymphatic depletion, infections, and death.<sup>42,67,68</sup> Thus, with rare exceptions<sup>84</sup> (Chapter 44), bone marrow transplants have been successful only when the donor has been an identical twin or an HL-A identical sibling of the recipient.<sup>42,126,128</sup> Even with an HL-A-identical sibling donor, GvH disease of variable severity may appear, presumably because of non-HL-A differences.<sup>42,128</sup> The histocompatibility typing

data should always be confirmed by mixed leukocyte cultures<sup>8</sup> (page 504). Successful allogeneic bone marrow transplants have been reported in patients with leukemia, those with aplastic anemia, and in persons with immune deficiency diseases of various forms.<sup>38,42,127,128</sup>

### Fetal-Maternal Relations

Antileukocyte antibodies are frequently found in sera from pregnant and multiparous women (page 504); their production appears to be triggered by the paternal contribution to the fetal transplantation antigens.

*Neonatal leukopenia* (Chapter 42) due to anti-HL-A antibodies must be exceedingly rare, although leukopenia apparently may result from antibodies directed against granulocyte specific antigens.<sup>57,58,59</sup> On the other hand, *neonatal thrombocytopenia* (Chapter 34) may occasionally be due to anti-HL-A antibodies, or, at other times, to antibodies directed against platelet specific antigens (page 512).<sup>114,120</sup> This appears to be a rare occurrence, however.<sup>115</sup>

A clearcut relationship between the presence of antileukocyte antibodies in the mother and fetal abortion, stillbirth, neonatal deaths, and congenital malformations has not been established,<sup>41,113</sup> although one study suggested an association between *congenital malformations* and the presence of maternal anti-HL-A antibodies.<sup>77</sup> The antibodies may, however, have been the result rather than the cause of the malformations.

*Gestational choriocarcinoma* is a unique malignant disease since it is of fetal origin and must therefore be considered an allogeneic graft. Thus, rejection should in most cases be expected and spontaneous regression, even when metastases are present, has been observed.<sup>30</sup> In keeping with this a correlation has been found between the degree of lymphocytic infiltration of the tumor and the chances of recovery.<sup>29</sup>

The quality of antitumor immunity in patients with choriocarcinoma must, at least in part, be a function of the histocompatibility

difference between the fetus (ie, the paternal haplotype) and the mother. Preliminary studies have indeed suggested that when husbands have haplotypes identical to those of the spouse, prognosis is poor, whereas it is much better when no haplotypes are shared.<sup>54</sup> In addition, fewer HL-A incompatibilities have been found in persons with generalized disease than in those having localized disease.<sup>73</sup> In other studies no such correlations have been found.<sup>110</sup> A restricted distribution of HL-A antigens may explain the 10- to 20-fold higher incidence of trophoblastic disease among Greenland Eskimos than among Caucasians; 81% of Greenland Eskimos were found to be HL-A9 positive, and some of the Four antigens were also noted to be much more frequently present in these people.<sup>74</sup> This implies a greater chance for Eskimo children to be HL-A compatible with their mother.

### Paternity Testing

The HL-A system now fulfills most of the requirements for use in the exclusion of paternity.<sup>82</sup> In general terms, a man is excluded if both he and the baby's mother lack an antigen that the baby has, or if antigens he has and must hand on are not present in the child.<sup>74</sup> It is possible that HL-A typing may eventually become more informative than erythrocyte typing.<sup>50</sup>

### Relation to Human Diseases

An association between histocompatibility antigens and susceptibility to a variety of diseases, including malignant lesions, has been clearly documented in animal systems.<sup>54</sup> In man, similar associations appear to exist. Those of hematologic interest include acute lymphoblastic leukemia,<sup>96,139,140</sup> chronic lymphocytic leukemia, and lymphosarcoma,<sup>89,140</sup> as well as Hodgkin's disease.<sup>2,36,140,143</sup> Such an association is of great interest in view of the well-established linkage between histocompatibility antigens and immune responses towards well-defined antigens in at least two animal systems.<sup>65</sup> Thus it is possible that the

association between susceptibility to childhood leukemia or other malignant disease and certain HL-A antigens has an immunologic basis.<sup>28</sup>

## Leukocyte Transfusions

Leukocyte transfusions are given under two circumstances: (1) in granulocytopenia when neutrophils are the desired cells and (2) in immune deficiency states (Chapter 44) when lymphocytes or their precursors are grafted in an attempt to establish normal immune responses. Lymphocytes have also been transfused as part of the experimental immunotherapy of tumors (Chapter 55).

The potential benefit of *granulocyte transfusions* has long been recognized in patients suffering from agranulocytosis and sepsis,<sup>196</sup> but the widespread clinical application of such therapy has been difficult to realize. This is due to several major problems: (1) Since the concentration of neutrophils in normal blood is approximately 1/1000 of that of red cells and 1/100 that of platelets, the collection of large numbers of cells is difficult. (2) The life span of the neutrophil in the blood is but a few hours, usually less than 10 (see Chapter 6). (3) The recovery of transfused neutrophils often is very poor, and the survival much less than 10 hours because of the presence of antileukocyte antibodies or the sequestration of cells in extravascular sites such as the liver and spleen. However, *improvements in collection techniques now* permit the routine harvest of large numbers of leukocytes from single donors.<sup>152,161,173,180,184</sup> and HL-A typing and cross-match techniques allow for better donor selection.<sup>170,174</sup> Under these circumstances, leukocyte transfusions result in a detectable increase in the number of circulating granulocytes and may lead to more effective control of infections.

### Indications

The routine use of platelet transfusions has made infection the leading cause of death in patients with hematologic malignant dis-

ease<sup>174</sup> (Chapter 54). While the introduction of the newer antibiotics has lowered the mortality rate of patients with gram-negative sepsis from 62 to 90%<sup>154,159 163 184,203</sup> to 29 to 38%,<sup>153 191</sup> the risk of death remains substantial both for gram-negative and other infections. Leukocyte transfusions may help to further decrease this risk.<sup>168 174 193</sup>

The most common indication for leukocyte transfusions is, therefore, bacterial sepsis that is unresponsive to appropriate antibiotic therapy and occurs in association with *severe leukopenia* due to primary disease of the marrow, or myelosuppression due to cytotoxic drugs. In addition, patients with granulocytopenia due to a variety of drug reactions (Chapter 41) and patients with aplastic anemia may benefit from granulocyte transfusions when they develop severe infections.<sup>194</sup> When neutropenia is due to increased rates of neutrophil destruction, as in congestive splenomegaly or in the presence of leukocyte antibodies (Chapter 42), leukocyte transfusions are probably without appreciable effect. Granulocyte transfusions may also be of benefit to patients suffering from *disorders of leukocyte function*, such as chronic granulomatous disease.<sup>190</sup> The induction of remissions following leukocyte transfusion in patients with leukemia has also been reported,<sup>151,179,202</sup> although leukemia does not, at present, constitute a valid indication for this form of treatment. The cause of such remissions is not clear, but graft-versus-host reactions<sup>192</sup> and feed-back inhibition<sup>158</sup> have been postulated as possible mechanisms.

### Selection of Donors

Potential cell donors should be free of disease that is transmissible by transfusion. The following are additional requirements: (1) The HL-A type of the donor and of the recipient should be determined. (2) Donors and recipients should be red-cell-type compatible and should have a negative red-cell cross-match (page 472). (3) Each recipient must be examined for the presence of antibodies against donor leukocytes. This can be done by leukocyte cytotoxicity<sup>72</sup> or leuko-

agglutination techniques.<sup>169</sup> If the recipient has antibodies against a potential donor, the latter's leukocytes should not be used since the intravascular sojourn of such cells is extremely brief. (4) Wherever possible, family members such as siblings and parents should be used as donors. Frequently, the need for leukocyte transfusions can be anticipated and leukocyte typing of family members will allow selection of the most histocompatible donor; the most desirable donor is, of course, a sibling with an identical HL-A phenotype (page 502).

### Collection of Granulocytes

#### From CML Donors

When more sophisticated techniques are not available, patients with chronic myelocytic leukemia in relapse are useful sources of large numbers of granulocytes,<sup>155 166,188</sup> since it is impractical to obtain adequate numbers of normal white cells without special techniques. Even under optimal conditions, 40 units of normal blood would have to be processed to obtain the same number of leukocytes recovered from one unit of leukemic blood.<sup>204</sup> It has been stated that leukemic leukocytes may not be as efficient as normal cells in fighting infection,<sup>182 190</sup> but this may be related in part to extracellular causes such as antileukocyte antibodies. Good intravascular survival, extravascular migration, and phagocytic function have been reported in recipients without preformed antibodies.<sup>166</sup>

CML leukocytes may be obtained by standard *leukopheresis techniques*, using plastic collecting bags and ordinary ACD anticoagulant. The buffy coat is obtained by differential centrifugation, the red cells are returned to the donor, and another unit of blood is obtained. Most donors tolerate leukopheresis of at least 2 or 3 units at a single sitting, but the procedure is time consuming. The collection of CML granulocytes by other techniques is discussed below. Since CML donors are almost invariably poor HL-A matches with the recipient, the effi-

ciency of such transfusions is reduced once antileukocyte antibodies develop (see below).

### From Healthy Donors

Improved collection techniques have now made it possible to recover adequate numbers of leukocytes from healthy donors and these are to be preferred whenever possible. The techniques of continuous flow centrifugation (CFC)<sup>152 153 173 184</sup> or filtration leukopheresis (FL, reversible leukoadhesion)<sup>161 176</sup> permit the collection of large numbers of normal granulocytes from a single donor. CFC allows for the rapid processing of blood (40 ml/min) and the collection efficiency is good: approximately one third of CML granulocytes and one fourth of normal donor granulocytes are recovered. At a single sitting,  $10^{10}$  normal cells and  $10^{11}$  leukemic cells are easily collected. Between 55 and 66% of these cells will be mature granulocytes.

In general, CFC is to be preferred to FL collections, but the FL technique is simpler and more readily available. CFC-collected leukocytes have normal intravascular survival ( $t_{1/2} = 6.5$  hours) and appear to function normally after transfusion.<sup>174</sup> In contradistinction, FL-collected leukocytes yield lower increments after transfusion, their hexose monophosphate shunt activity and bactericidal capacity are somewhat reduced *in vitro*, and they may well have abnormal intravascular survival patterns.<sup>174,180</sup> In addition, most recipients of FL-collected leukocytes develop chills and fever after transfusion, even though donors are carefully selected.<sup>174</sup>

### Transfusion of Granulocytes

Following collection, granulocytes should be transfused as quickly as possible. Employing the number of granulocytes available from single histocompatible donors (about  $10^{10}$ ) it is possible to achieve median one-hour transfusion increments of 250 to 850 granulocytes/ $\mu$ l/ $m^2$  of body surface.<sup>175</sup> However, the percent recovery of leukocytes

one hour after transfusion is closely linked to the goodness of the match. Median recovery of HL-A and ABO identical leukocytes is 50%, but there is a progressive decrease as the number of mismatched HL-A antigens increases—28% with one mismatch, 17% with two mismatches, and only 3% with three mismatches—even in the absence of demonstrable leukoagglutinins.<sup>171</sup> When leukoagglutinins are present, the recovery is very low. In addition, the recovery of transfused leukocytes is compromised when HL-A matched but ABO mismatched leukocytes are transfused (7%). The importance of HL-A compatibility also is reflected in the poor recovery of CML leukocytes (median 5%),<sup>169 186,192</sup> both in the presence (<1%) and absence (15%) of preformed antibodies.<sup>169 170 188</sup> Patients with preformed antibodies may have severe transfusion reactions and bacterial killing may be reduced.<sup>169 170 188</sup> Antileukocyte antibodies are most often the result of multiple transfusions but may also follow pregnancy, or organ transplantation; occasionally their presence is unexplained.

Because transfusion of viable lymphocytes into immunosuppressed patients may lead to accidental engraftment and an ensuing graft-versus-host disease,<sup>171</sup> most clinicians prefer to irradiate the leukocytes with 1,500 rads before transfusion.<sup>174</sup> It is possible, however, that irradiation may impair the phagocytic function of neutrophils (Chapter 42).

Whenever possible, granulocyte transfusions should be repeated daily until the infection is under control; a progressive improvement in patient survival has been reported with increasing numbers of transfusions.<sup>174,175</sup> Significant benefit from leukocyte transfusions has been claimed in reports of several studies,<sup>168,193</sup> although accounts of strictly randomized trials have not yet been published. It would seem reasonable, however, on the basis of available evidence, to support septic patients who are leukopenic with well-matched granulocyte transfusions wherever these are available.



## Complications

Complications range from mild acute reactions such as *fever* and *chills* to severe reactions with *respiratory distress*, retrosternal constriction, pallor, and cyanosis. Respiratory distress is presumably due to sequestration of agglutinated leukocytes within the pulmonary microvasculature.<sup>164,176</sup> Transient *pulmonary infiltrates* may also occur in the presence of leukoagglutinins.<sup>201</sup> Several types of infections have been transferred by leukocyte transfusions, including malaria<sup>162</sup> and toxoplasmosis<sup>193</sup>; serum hepatitis has not been clearly documented, but undoubtedly occurs.

## Platelet Antigens

Several systems of antigenic determinants are found on the surface of platelets.<sup>269</sup> These include (1) the HL-A antigens, which are shared with leukocytes and other tissues; (2) ABO antigens, which are shared with red cells; and (3) platelet-specific antigens. All of these antigens are detected by means of isoantibodies formed after transfusion or pregnancy. In addition, immunization of dogs, rabbits, guinea pigs, and chickens with human platelets yields heterologous anti-platelet antisera,<sup>233,234,264</sup> but the specificities detected by these have not been further delineated and their clinical significance is unknown. They will not be discussed further.

### HL-A Antigens

In 1962, Shulman and coworkers studied sera from two women whose babies suffered from neonatal thrombocytopenia and found that these sera reacted with platelets from 49 normal individuals. The corresponding antigen was called Pl<sup>B1</sup> (later PlGrLy<sup>B1</sup>) and appears to be inherited as a dominant character in the two families studied. Later this antigen was found to be the same as the histocompatibility antigen Mac (= HL-A2).<sup>45</sup> Subsequently, other histocompatibility antigens were shown to be present on platelets and now all currently known LA (First

series) antigens and most Four (Second series) antigens (Table 12-1) are known to be represented.<sup>269</sup> It seems reasonable to suggest that eventually all HL-A isoantigens will be detectable on platelets. Indeed, platelet typing represents a simple and reliable alternative to leukocyte typing, even for purposes of organ transplantation (page 514). The most important older designations for platelet antigens and their current HL-A equivalents are listed in Table 12-3.

Each platelet contains approximately one fifteenth to one fifth the amount of antigen found on leukocytes.<sup>257,264,268</sup> A dosage effect has been shown for at least one antigen; the platelets of homozygous HL-A2 family members carry approximately twice as much antigen as do the platelets from heterozygous individuals.<sup>264</sup> It has also been shown that the amount of HL-A2 antigen present on the platelets of unrelated HL-A2 subjects is influenced by other genetic determinants belonging to the LA (First) series, but not by those belonging to the Four (Second) series.<sup>269</sup>

### Other Isoantigens Shared by Platelets and Leukocytes

In addition to the HL-A antigens, platelets and leukocytes also share the 5a/5b antigens, a presumably diallelic system first described by van Leeuwen.<sup>242,260</sup> The 5a/5b characters segregate independently of the HL-A system.

**Table 12-3. Platelet Antigen Designations and Their Histocompatibility Antigen Equivalents**

Platelet Antigen	Histocompatibility Antigen Equivalent
Pl <sup>B1</sup>	HL-A2
Pl <sup>B2</sup>	HL-A8
Pl <sup>B3</sup>	HLA1 and 7
PlGrLy <sup>B1</sup>	HL-A2
PlGrLy <sup>C1</sup>	7 <sup>a</sup>
PlGrLy <sup>F1</sup>	Oa 14†

<sup>a</sup>Van Rood nomenclature

†Dausset nomenclature

Their presence on platelets is only detectable by absorption studies, a rather indirect technique.<sup>211,212</sup> The 9a antigen as well as the Na1, NB1, and Vaz antigens apparently are only found on leukocytes<sup>260</sup> (page 506).

### ABO Antigens

A and B antigens have been demonstrated on the surface of platelets by a variety of techniques<sup>269</sup> including adsorption experiments,<sup>253-277</sup> agglutination<sup>231,213,215</sup> and mixed cell agglutination studies,<sup>220-243</sup> complement fixation,<sup>209</sup> and platelet survival studies.<sup>210-238</sup> Whether A and B antigens are actually intrinsic parts of platelet membrane structure<sup>253</sup> or simply are adsorbed<sup>243</sup> remains unresolved, it is of interest that group O platelets become firmly coated by soluble A and B substances added *in vitro*.<sup>241</sup> From a practical point of view it should be noted that the transfusion of ABO incompatible platelets results in lower platelet levels than the transfusion of ABO compatible ones, although the survival time of a sub-population of these ABO incompatible platelets appears to be normal.<sup>210-255</sup> Nevertheless, some workers believe that ABO antigens are either totally absent from platelets or present in very small amounts.<sup>214-261</sup>

Other red cell antigens have not been convincingly demonstrated on platelets,<sup>278</sup> although claims have been made for the presence of *Rh* antigens,<sup>245,246</sup> among others.

### Platelet-Specific Isoantigens

In addition to antigens that platelets share with other cells there are those that appear to be restricted to platelets. Of these, DUZO was the first to be described in detail; others include the  $PI^{A1}$  system, the Ko system, and the  $PI^E$  system (Table 12-4).

### DUZO

The antibody defining DUZO was discovered in the serum of a French mother, Madame Duz., whose four children had died of neonatal purpura.<sup>259</sup> The platelets from 18 of 82 (22%) randomly selected Frenchmen also gave strong reactions with this antiserum. The antigen appears to be absent from red cells and white cells.<sup>252</sup> Later the antibody was described in one other individual.<sup>251</sup> It is not known whether DUZO is related to any of the other platelet-specific antigens.<sup>269</sup>

### $PI^A$ (Zw)

The antibody defining the first antigen of the  $PI^{A1}$  system was described independently by Van Loghem and associates ( $Zw^a$ )<sup>244</sup> and by Shulman and coworkers ( $PI^{A1}$ )<sup>263</sup> who showed that the platelets of about 98% of unrelated individuals readily reacted with it. The identity of the two antigens was established later.  $PI^{A1}$  appears to segregate as a dominant character and clearly shows a dos-

Table 12-4. Platelet Specific Antigens and Their Respective Gene Frequencies

System	Antigen	Gene Frequency	Reference
DUZO	DUZO	?	250
$PI^A$ (Zw)	$PI^{A1}$ (Zw <sup>a</sup> )	0.84 (Dutch)	244
		0.83 (N. American)	265
		0.144 (Dutch)	278
		0.074 (Dutch)	278
Ko	Ko <sup>a</sup>	0.920 (Dutch)	278
		very common (N. American)	265
$PI^E$	$PI^{E1}$	0.025 (N. American)	265

After Svegaard <sup>269</sup> courtesy of the author and Series Haematologia

age effect.<sup>264</sup> The antigen is only found on platelets,<sup>280</sup> including those of all monkeys tested, the dog, and the rabbit.<sup>264</sup> Anti-PI<sup>A1</sup> (Zw<sup>a</sup>) antibodies have been found in cases of isoimmune neonatal thrombocytopenia due to maternal isoimmunization,<sup>264</sup> following multiple transfusions, and in individuals suffering from post-transfusion purpura (Chapter 34).<sup>261,269</sup>

Subsequently the product of a gene allelic to PI<sup>A1</sup> (Zw<sup>a</sup>) was described. The antibody defining this allelic specificity (PI<sup>A2</sup> or Zw<sup>b</sup>) was recovered from a patient who had received many transfusions<sup>280</sup> and was shown to react with about 28% of predominantly group O platelets from Dutch individuals.<sup>278</sup> The antigen is only found on platelets and has so far been restricted to those of *Homo sapiens*.<sup>278</sup> Like its allele, PI<sup>A2</sup> (Zw<sup>b</sup>) is inherited as a meelian dominant character. Approximately 69% of Dutch and North American individuals are homozygous for PI<sup>A2</sup>, 3% are homozygous for PI<sup>A2</sup>, and 28% are heterozygotes. The PI<sup>A</sup> (Zw) system does not appear to be closely linked to the ABO, Rhesus, MNS, Duffy, or Ko (see below) systems.<sup>278</sup>

## PI<sup>E</sup>

The PI<sup>E</sup> system appears to contain two allelic antigens, PI<sup>E1</sup> and PI<sup>E2</sup>. The antibodies defining these antigens were originally recovered from a patient who had received many transfusions and from the mother of a baby who had neonatal thrombocytopenia, respectively.<sup>264</sup> The antibodies readily bind complement. Anti-PI<sup>E1</sup> reacts with the platelets from 99.9% of all individuals tested, whereas anti-PI<sup>E2</sup> reacts with only 5%; thus, virtually all PI<sup>E2</sup> individuals must be heterozygotes, and less than 0.1% of the population have been calculated to be homozygous for PI<sup>E2</sup>. PI<sup>E2</sup> appears to be inherited as a dominant character<sup>264</sup> and, while the same probably is also true for PI<sup>E1</sup>, the required criteria for dominant inheritance<sup>259</sup> have not yet been satisfied. The independence of the PI<sup>E</sup> and Ko systems (see below) has not yet been

demonstrated.<sup>269</sup> Platelets from all primates react with anti-PI<sup>E1</sup> but not with anti-PI<sup>E2</sup>.<sup>269</sup>

## Ko

The Ko system is characterized by at least two antigens, Ko<sup>a</sup> (initially called Ko)<sup>279</sup> and Ko<sup>b</sup>.<sup>247,269,278</sup> Ko<sup>a</sup> is found on the platelets of about 13 to 17% of Europeans tested, whereas Ko<sup>b</sup> is almost universally present (> 99% in Holland) (Table 12-4). Both of these antigens are inherited as dominant characters and their allelic relationship has been demonstrated in family studies.<sup>278</sup>

Anti-Ko<sup>a</sup> and anti-Ko<sup>b</sup> antibodies appear to be encountered most commonly in platelet agglutinating sera.<sup>278</sup> Anti-Ko<sup>b</sup> sera behave strangely in absorption studies, however, since the constituent antiplatelet antibody can be absorbed by Ko<sup>b</sup> - (ie, Ko<sup>a</sup>) platelets as well as by Ko<sup>b</sup> + platelets. This may be due to cross-reactivity between Ko<sup>a</sup> and Ko<sup>b</sup> or to the presence of small amounts of Ko<sup>b</sup> antigen in Ko<sup>a</sup> individuals.<sup>278</sup>

The Ko system is not linked to the ABO, Rhesus, Duffy, or Se systems.<sup>221,278</sup> Anti-Ko antibodies apparently do not cause neonatal thrombocytopenia.<sup>230,269</sup>

## Methods of Detection

The two most commonly used methods of platelet typing are thromboagglutination and complement fixation. In the *thromboagglutination test*, platelets are incubated with platelet antibody (or test serum) and the mixture is then examined under the microscope for agglutination. A large number of modifications of this basic test have been described,<sup>222,235,245,274,278</sup> no doubt reflecting the difficulties inherent in the technique. The greatest problem is the natural tendency of platelets to clump even in the absence of antibodies, and this makes interpretation of test results difficult. Attempts have been made to eliminate these nonspecific reactions by the use of EDTA as the anticoagulant,<sup>222,245,278</sup> and by heating<sup>222,245</sup> or washing<sup>270</sup> the platelets. One

of the most successful modifications appears to be that of van der Weerdt,<sup>278</sup> in which platelets are washed in EDTA buffer at 4°C and are kept at this temperature for some time to allow platelet clumps to settle out.

Thromboagglutination appears to be particularly useful for the detection of platelet-specific isoantigens, especially those belonging to the PI<sup>A</sup> (Zw) and Ko systems, which are rarely, if ever, detected by other techniques.<sup>269</sup>

The complement-fixation technique is unsurpassed for HL-A typing of platelets<sup>218,229,261,272</sup> as well as for the platelet specific isoantigens PI<sup>E1</sup> and PI<sup>E2</sup>.<sup>261</sup> Shulman's quantitative technique<sup>261</sup> was later simplified by Aster.<sup>212</sup> The usefulness of this modified technique has been confirmed by a large number of studies.<sup>228,239,249</sup> In one version of this method,<sup>271</sup> 40  $\mu$ l of platelet suspension ( $300 \times 10^9$  platelets/l of 0.1% sodium azide in saline), 40  $\mu$ l of inactivated test serum (56°C for 30 minutes), and 40  $\mu$ l of C' dilution are mixed in a test tube and incubated at 37°C for 60 minutes. The concentration of guinea pig or human complement is adjusted to give 80 to 90% hemolysis in a control. At the end of the incubation period, 100  $\mu$ l of a 3% suspension of antibody-sensitized sheep red cells and 500  $\mu$ l of C' buffer are added and incubated for an additional 60 minutes. The amount of hemolysis seen at this time reflects the fixation of complement by platelet-antiplatelet antibody complexes; no hemolysis indicates complete fixation of complement and therefore the presence of antiplatelet antibodies, whereas hemolysis indicates the opposite. Appropriate controls for the absence of anticomplementary activity in the system as well as to prove the activity of the complement should always be included.<sup>271</sup> With good sera, the reproducibility of the C' fixation test is equal to that of the lymphocytotoxicity test, both of which are in excess of 99%. In addition, platelets are more easily stored than lymphocytes and the C' fixation test is suitable for quantitative purposes.<sup>225,261,271</sup> The test has also been modified to allow the detection of non-complement-fixing (blocking) antibodies<sup>264</sup>

including those found in the serum of some patients with rheumatoid arthritis.<sup>238</sup>

A microtechnique for HL-A typing of platelets by complement fixation requires only 2  $\mu$ l of all test reagents instead of the customary 40  $\mu$ l and promises to be just as reproducible and reliable as the macro method.<sup>217,219</sup> Because of the stability of platelet suspensions, in the future this test may replace the lymphocytotoxicity test for routine histocompatibility testing.

Other tests for the detection of platelet antigens and antiplatelet antibodies include absorption studies,<sup>242,253,257</sup> the antiglobulin consumption test,<sup>229,250,267</sup> mixed cell agglutination,<sup>220,243</sup> clot retraction inhibition,<sup>263,282</sup> platelet factor 3 release,<sup>237</sup> and platelet survival studies.<sup>211,216</sup>

### Nature of Antiplatelet Antibodies

In general, antibodies detected by their complement-fixing properties are IgG, whereas isoagglutinins are IgM.<sup>262</sup> It has been estimated that between 500 and 1,000 antibody molecules per platelet are sufficient to bring about platelet destruction in vivo.<sup>264</sup> This amount of antibody will only occupy about 1% of the total number of antigenic sites on the platelet surface and is too small to be detected by any of the above-mentioned in vitro tests.

### Clinical Significance of Platelet Antigens

Platelet antigens are of clinical significance as transplantation antigens, as a cause of neonatal thrombocytopenia, and as important factors in the success or failure of platelet transfusion therapy.

### Transplantation

Since platelets and other tissues share surface antigens (page 511) it is not surprising that tissue grafts lead to shortened survival of platelets carrying the same antigens.<sup>215,216</sup> Conversely, platelet transfusions may lead to a shortened survival of subsequently applied

tissue grafts.<sup>224</sup> Nevertheless, while platelets have a surface that is about one tenth that of a lymphocyte and the amount of surface antigen per platelet is proportionately reduced,<sup>224,264,269</sup> their immunogenicity is much less than can be explained by differences in size and weight alone.<sup>224</sup> It has been shown, for instance, that 1,000 times more platelets than leukocytes have to be injected in order to induce a second-set skin-graft response.<sup>223</sup> The reason for this difference in immunogenicity is not understood.

### Fetal-Maternal Relations

Isoimmune neonatal purpura<sup>269</sup> is the counterpart of red cell hemolytic disease of the newborn and is discussed in detail elsewhere (Chapter 34). Current serologic techniques reveal platelet antibodies in only half of all suspected cases. The antigens identified in isoimmune neonatal purpura have included the platelet-specific antigens DUZO,<sup>250</sup> PIA<sup>1</sup>,<sup>264</sup> and PIE<sup>1</sup>,<sup>264</sup> as well as several HL-A antigens.<sup>264,273,278</sup> PIA<sup>1</sup> is implicated most frequently,<sup>265</sup> but only when the blocking test is applied, since anti-PIA<sup>1</sup> antibodies do not bind complement. Among the HL-A antigens, HL-A2 is most often responsible.<sup>265</sup>

## Platelet Transfusion

When hemorrhage is due to thrombocytopenia, platelet transfusion is not only practical but frequently it is lifesaving. In most instances the success or failure of such therapy depends on the functional integrity of the transfused platelets, the cause of the platelet defect in the recipient, and the presence or absence of platelet antibodies. Unfortunately the selection of donors is still rather unsophisticated, since routine matching for histocompatibility is not feasible. All of these aspects of platelet transfusion will now be considered in turn.

### Collection and Preservation

Platelets may be transfused in the form of platelet-rich plasma (PRP), platelet concen-

trates (PC), or, occasionally, as fresh whole blood. Blood is collected in conventional ACD-A anticoagulant (Chapter 11) by means of a triple or quadruple pack unit to allow closed handling of the pack's contents. Initially the blood is kept at room temperature and is centrifuged within an hour of collection, usually at 1500 g for six minutes, also at room temperature. The supernatant PRP can then be separated within the closed system. It contains between 75 and 90% of the original number of platelets in the unit. If the blood comes from a routine donor, the resultant packed red cells are stored for use in transfusion (Chapter 11), but if the platelets are obtained by the process of plasmapheresis, then the red cells are immediately returned to the donor and a second unit of blood is obtained. In the latter instance, between 2 and 4 units of blood (1000 or 2000 ml) may be processed at a single sitting, but if more than 2 units are obtained, it is necessary to return about half the platelet-poor plasma following the preparation of platelet concentrates. A donor may give 4 units of platelets each week without experiencing depletion.<sup>309</sup>

In most instances, PRP is further processed to yield platelet concentrates (PC). The PRP is centrifuged at 3000 g for 10 minutes; this concentrates about 95% of available platelets into a button. All but 30 ml of the supernatant platelet-poor plasma is expressed into a third side bag, and may be processed further to yield cryoprecipitate and other plasma components, or it may be returned to the donor. When platelets are used within 24 hours of collection, storage of the platelet button at room temperature will generally make it easy to resuspend platelets without excessive clumping,<sup>313</sup> but a preparation particularly free of aggregates results if the platelets are stored on a slowly rotating disc that is tilted at an angle of 30 degrees to the horizontal.<sup>289</sup> When centrifugation is performed at lower than room temperature, acidification of the PRP has been recommended in order to prevent irreversible clumping of platelets,<sup>288,290</sup> but at room temperature this is not necessary.<sup>289</sup> In addition,

acidification of PRP diminishes the yield of cryoprecipitate from platelet-poor plasma,<sup>288</sup> and, more importantly for present purposes, diminishes the viability of platelet concentrates stored at room temperature.<sup>251</sup>

A number of important biochemical and functional changes occur in platelets during storage, depending in part on the duration and the temperature of storage. Thus, platelets kept at 22°C show a continuous accumulation of lactate, and a dramatic fall in platelet glycogen.<sup>320,321</sup> Total ATP pools do not appear to change markedly either at 22°C or 4°C,<sup>316,320,321</sup> but metabolic ATP is reduced by about half within 24 hours at 4°C, with a corresponding increase in hypoxanthine production.<sup>316</sup> The loss of metabolic ATP is at least partially reversible by inosine.<sup>316</sup> In addition, stored platelets quickly lose their ability to be aggregated by ADP, epinephrine, collagen, and agar.<sup>298,312,321,336</sup> This defect is more marked with storage at 22°C than at 4°C.<sup>336</sup> It also appears to be more severe when platelets are stored as concentrates, rather than as PRP.<sup>312</sup> After transfusion into thrombocytopenic patients, recovery of platelet glycogen and the capacity to be aggregated reappear within 24 hours,<sup>312,321</sup> but this delay may be of crucial importance in a critically ill patient. Glucose utilization through glycolysis, the hexose monophosphate shunt, and the tricarboxylic acid cycle seem to remain intact.<sup>321</sup>

Storage conditions also affect the function and survival of platelets after transfusion. When the <sup>51</sup>chromium technique is used, the yield and survival of autologous platelets stored at 22°C for relatively short periods of time (<24 hours) appear to be much superior to those of platelets stored at 4°C,<sup>296,319</sup> but only if the pH of platelet concentrates is maintained above 6.3.<sup>296</sup> However, when isologous *unlabeled* platelets are given to thrombocytopenic patients after 48 to 72 hours of storage, the yield and survival of platelets are best if the platelets have been stored in the cold.<sup>295,296</sup> In addition, platelets kept at 4°C for 24 to 72 hours are superior to those kept at 22°C with respect to their capacity to shorten the bleeding time and

effect hemostasis, both in patients with thrombocytopenia and in those with platelet defects due to aspirin ingestion.<sup>295,296</sup> The relative ineffectiveness of platelets stored at room temperature for prolonged periods appears to be the result of the more rapid onset of metabolic and functional defects under those conditions (see above). Thus, platelets stored for more than 24 hours prior to transfusion should be refrigerated at 4°C. If platelets are given within 24 hours of collection, storage at ambient temperatures does not adversely affect their function.<sup>296</sup>

Like red cells, platelets may be preserved in the frozen state with the help of *cryoprotective agents* such as glycerol, dimethylsulfoxide (DMSO), dimethyl- and diethylacetamide, and hydroxyethyl starch.<sup>299,301,308,312a,333,331</sup> Frozen platelets should be of great potential value in disaster areas, for shipment to remote places, and for the establishment of autologous depots to be stored before patients undergo intensive chemotherapy or radiotherapy. Unfortunately, cost considerations, possible side effects of cryoprotective agents, and the known loss of viability of frozen platelets have limited the usefulness of cryostorage thus far.<sup>294</sup>

## Matching Procedures

### ABO Antigens

Since A and B antigens are represented on platelets it would seem prudent to transfuse only ABO compatible preparations. Shortened platelet survival is noted when A platelets are transfused into O recipients.<sup>287</sup> Some centers, however, do not consider ABO incompatibility to be of major importance in the treatment of thrombocytopenic patients and ignore ABO types in platelet transfusion therapy.<sup>294</sup> In our present state of knowledge, transfusion of ABO incompatible preparations would appear to be unwise, if avoidable.

### Rh Antigens

Although the evidence for the presence of Rh antigens on platelets is not convincing,

Rh compatibility should be observed when platelets are given to young females suffering from curable or chronic diseases, since red cells invariably contaminate platelet preparations. If Rh incompatibility cannot be avoided and sensitization to Rh antigens is of major concern, anti-Rh immunoglobulin may be given prophylactically (Chapter 27), as in Rh-incompatible pregnancies. In all other situations, Rh typing is unnecessary in platelet transfusion therapy. The risk of Rh sensitization is markedly decreased in immunosuppressed patients.<sup>307</sup>

### Platelet-Specific Antigens

The  $PI^A$  (Zw),  $Ko$ , and  $PI^E$  systems are specific to platelets, but of these only  $Ko^a$  leads to transfusion-induced isoantibodies with a clinically relevant frequency.<sup>270</sup>  $Ko^a$  is mismatched in only 13% of random blood transfusions and when a  $Ko^a$ -negative individual forms anti- $Ko^a$  antibodies, only  $Ko^a$ -negative platelets should be given.

### HL-A Antigens

HL-A antigens constitute by far the most important system of antigens on platelets. They induce antibody formation more readily than do platelet-specific antigens and can greatly diminish the survival time of HL-A incompatible platelets. Sensitization follows repeated platelet transfusion, leukocyte transfusion, tissue transplants, or, most commonly, routine blood transfusion. In virtually all of these, a mismatch for one or more antigens belonging to the HL-A system occurs. Unfortunately, it is impossible to prevent this complication of transfusion therapy in most instances, since it has been estimated that in excess of 20,000 different HL-A phenotypes are possible, and even the most common phenotype (HL-A 1,3,7,8) has a frequency of only 1% in Caucasians.<sup>270</sup> Thus, unless a sibling is to serve as a regular platelet donor, it is presently impractical to match platelets for HL-A antigens routinely, except in major centers where large donor panels are readily available.<sup>173</sup> Platelets from persons having an

almost perfect match are tolerated no better than those from random donors.<sup>339</sup>

The following scheme of donor-recipient matching is employed by most laboratories: (1) Nonimmunized patients are given platelets without regard to HL-A typing. In order to reduce the risk of isosensitization, the number of platelet transfusions should be kept to the lowest quantity that will meet the needs of the patient. (2) If the recipient has siblings, they should always be HL-A typed, since there is a statistical probability that 25% of these will be HL-A identical with the patient. When HL-A identical siblings are available, they are the donors of choice.<sup>309,339</sup> (3) When the yield and survival of transfused platelets are unexpectedly poor, the patient should be HL-A typed and the specificity of the antibody should be established. When antibodies of limited specificity are found, it may be possible to match for platelets not carrying the corresponding antigens. Alternatively, when sibling donors are not available, unrelated compatible donors may be selected by HL-A matching.<sup>340</sup>

### Administration

Platelet concentrates usually are delivered in plastic bags. The contents of several of these are withdrawn into a large plastic syringe and injected into the patient through an appropriate recipient set containing a filter.<sup>286</sup> Because platelet concentrates come with a minimum of plasma (usually 30 ml or less) a relatively large number of platelets remain in the bag. These should be recovered by adding a small amount of saline solution to the contents of the bag. The time interval between phlebotomy of the donor and the injection of platelets into the recipient should be kept as short as possible, the optimum being less than six hours. This will assure superior platelet survival and function.

### Dosage of Platelets

Unless the destruction of transfused platelets is unduly rapid, the increment in circulating platelets will be  $10$  to  $12 \times 10^9/l/m^2$

of body surface for every unit of platelets given (usually  $10^{11}$  platelets per unit).<sup>307</sup> Thus, in a child weighing 30 kg and having a surface area of about  $1 \text{ m}^2$  a platelet rise of  $20$  to  $25 \times 10^9/\text{l}$  could be anticipated following administration of 2 units of platelets. Since the actual yield varies considerably from patient to patient, and from situation to situation for any given patient, it is important to assess yield and survival after platelet transfusions. In order to determine yield, platelet counts are made shortly before and one hour after transfusion. Post-transfusion counts made at 5, 30, 60, and 180 minutes furnish a valuable index of platelet survival in patients with increased platelet turnover rates. When these counts are plotted on semi-logarithmic paper, a rough but useful survival time ( $t/2$ ) may be calculated. More elegant techniques utilizing  $^{51}\text{chromium}$ -labeled platelets are available in some laboratories.<sup>296,319</sup>

The frequency of transfusions will depend on the half-life of transfused platelets, which in most thrombocytopenic patients without antibodies is about two days or less.<sup>307</sup> The half-life of autologous platelets in normal individuals is, of course, much longer (Chapter 9). The platelet count should be followed daily and subsequent transfusions should be guided by changes in these counts and other clinical considerations (see below).

### General Indications

Platelet transfusions are given to stop or prevent bleeding due to thrombocytopenia or abnormalities in platelet function. The critical platelet level at which bleeding occurs varies and depends on (1) the cause of the thrombocytopenia; (2) the presence of concurrent disease such as sepsis, immune vasculitis, or infiltrative diseases such as leukemia; (3) the presence of other clotting defects, as in von Willebrand's disease, disseminated intravascular coagulation, or vitamin K deficiency; and (4) exposure to drugs such as aspirin or others that interfere with the function of the remaining platelets (Chapter 35). The average age of the circulating platelets

also is important, since newly released platelets appear to function more effectively than older ones.<sup>306</sup> This may explain, at least in part, why, at similar platelet levels, patients with thrombocytopenia due to immune destruction tend to bleed less than those with defects in platelet production.<sup>306</sup>

If the function of individual platelets is adequate, life-threatening hemorrhage usually does not occur until the platelet count is less than  $20 \times 10^9/\text{l}$ . Many patients, especially those with thrombocytopenia due to increased destruction, may have no bleeding episodes for long periods of time, even though the platelet count is as low as  $5 \times 10^9/\text{l}$ . However, for patients with other illnesses such as leukemia, the threat of serious hemorrhage has been estimated to be in excess of 1%/day for counts of  $20 \times 10^9/\text{l}$ , with a rise to 20% when the count drops to  $1 \times 10^9/\text{l}$ <sup>305</sup> (Chapter 54). The risk of hemorrhage is particularly great in the presence of fever and infection, or when thrombocytopenic patients are inadvertently exposed to drugs interfering with platelet function. When serious hemorrhage develops, platelets must be transfused as soon as possible and this must be continued until the bleeding is under control.

Indications for prophylactic platelet transfusions are less clear; in general, they are contraindicated in immune thrombocytopenias and in conditions of chronic marrow failure such as aplastic anemia, but are probably indicated in patients with bone marrow suppression due to chemotherapy (see below for details).

### Thrombocytopenias Due to Decreased Production

#### Leukemia (Chapter 54)

Although the frequency of death due to hemorrhage has dropped dramatically with the advent of repetitive platelet transfusions,<sup>311,313</sup> hemorrhage still constitutes the second most common direct cause of death and is undoubtedly a complicating factor in many patients dying from infection. In par-



ticular, the risk of intracranial hemorrhage increases sharply when platelets drop below  $20 \times 10^9/l$ .

Indications<sup>303</sup> for platelet transfusions in leukemia include (1) any overt hemorrhage such as persistent epistaxis and gingival bleeding, or gross hematuria, hematemesis, or melena; (2) intracutaneous hemorrhages and particularly *fresh* crops of petechiae; (3) expanding intramuscular hematomas; and (4) suspected or proven internal bleeding, including cerebral hemorrhage.

*Prophylactic platelet transfusions* are probably indicated in acute leukemia when counts drop below  $10 \times 10^9/l$ . At these levels the risk of hemorrhage is high and the platelet count should therefore be maintained above this figure whenever possible.<sup>303</sup> Patients with  $20 \times 10^9$  or more platelets/l usually do not need prophylactic transfusions unless the counts are dropping rapidly or there is fever, infection, or mucosal ulceration. If a leukemic patient has to undergo a surgical procedure, he should receive enough platelets to raise the count to  $100 \times 10^9/l$  or more.<sup>303</sup>

It is said that isoimmunization and refractoriness to further platelet therapy develop more slowly in patients with leukemia than in those with aplastic anemia, perhaps because of the concomitant use of highly immunosuppressive chemotherapeutic agents.<sup>304</sup> Nevertheless, sensitization does occur<sup>337</sup> and when ordering platelet transfusions it is well to remember that platelet antibody production is at least in part a function of the number of transfusions received. The risk of sensitization is reduced greatly when platelets from histocompatible siblings are used (page 517).

### *Marrow Failure*

Hemorrhage secondary to thrombocytopenia is a common cause of death in patients with aplastic anemia (Chapter 56). Since marrow aplasia may persist for many years, it is imperative that blood and platelet transfusions, especially those from random donors, be given only when absolutely necessary, as isoimmunization will inevitably occur and

make further platelet transfusions ineffective.<sup>309</sup> A number of transfusions (perhaps 15 to 20) are ordinarily required before sensitization becomes evident and prudent restraint will not only delay the appearance of antiplatelet antibodies, but may very well extend the patient's life expectancy. Again, histocompatible siblings are to be preferred to random donors.<sup>339</sup> Because of the prolonged clinical course, prophylactic platelet transfusions are usually not feasible in patients with chronic marrow failure.

When platelets are tagged with very small amounts of antibody, sequestration occurs predominantly in the spleen, whereas larger amounts of antibody lead to hepatic destruction.<sup>291</sup> Splenectomy seems to have resulted in improved platelet survival and decreased transfusion requirements in some patients with aplastic anemia or leukemia.<sup>175,302,309</sup> Benefits are most marked when histocompatible platelets from siblings or unrelated donors are available,<sup>175,309</sup> but in rare instances platelet recovery is also increased when platelets of nonmatched unrelated donors are used.<sup>175</sup>

## **Thrombocytopenias Due to Increased Destruction or Utilization**

### *Immune Destruction*

**ACUTE IDIOPATHIC THROMBOCYTOPENIC PURPURA (ITP).** This disorder is most common in children and is, in most instances, self-limited (Chapter 34). Serious bleeding is uncommon and platelet transfusions are rarely necessary. When platelets are given they are rapidly destroyed, but steroid therapy may sometimes result in improved survival. In chronic ITP the survival of transfused platelets is also very short and under most circumstances it is useless to expose such patients to the risk of multiple transfusions.

**NEONATAL ISOIMMUNE THROMBOCYTOPENIC PURPURA.** Newborns suffering from thrombocytopenic purpura due to maternal antibodies directed against platelet antigens

inherited from the father<sup>269,287</sup> are best transfused with maternal platelets that have been washed in compatible AB serum so as to prevent further transfer of maternal antiplatelet antibody into the baby.<sup>285</sup> The washing procedure is not necessary if histocompatible donors lacking the antigen in question are available, whether this be a platelet-specific or an HL-A antigen. In many instances, maternal siblings constitute the best source of potential platelet donors. The baby's father should *never* be used as a platelet donor.

**DRUG-INDUCED IMMUNE THROMBOCYTOPENIA** (Chapter 34). In most instances of drug-induced immune thrombocytopenia, the majority of which are of the quinine or "innocent bystander" variety, transfused platelets are destroyed rapidly and it is unlikely that platelet transfusions are warranted under such circumstances unless most of the drug has been eliminated.<sup>294,317</sup> Severe febrile reactions may occur.<sup>294</sup>

#### *Nonimmune Destruction or Utilization*

**DISSEMINATED INTRAVASCULAR COAGULATION (DIC)** DIC is one of the most common causes of thrombocytopenia (Chapter 38). Bleeding is not solely due to thrombocytopenia; other causes include depletion of labile clotting factors, vascular damage, and the poor functional state of available platelets. Thus platelet transfusions are unlikely to be of benefit unless the whole process of intravascular coagulation is brought under control. When this is done by appropriate therapy (Chapter 38), platelet transfusions may play a useful role by protecting the patient from hemorrhage due to thrombocytopenia, especially if the patient is receiving heparin. However, in the absence of these definitive measures, platelet transfusions are probably not indicated and may indeed be dangerous as they will undoubtedly participate in further intravascular coagulation.

**MASSIVE TRANSFUSIONS** (Chapter 11). The platelets of patients receiving massive transfusions of stored blood may be washed out of the circulation and thrombocytopenia may

result. Most commonly this occurs following massive gastrointestinal hemorrhage, during major vascular surgical procedures, or in patients with hemorrhagic tendencies who have been inadequately prepared for surgical operations. When the equivalent of one whole blood volume (5,000 ml in a 70-kg man) is replaced with one-day-old bank blood, the platelet count will usually drop to about  $50 \times 10^9/l$ , but lower levels may be reached if more massive transfusions are given. When this occurs, platelet transfusions and/or the exclusive use of absolutely fresh blood are indicated.

A similar depletion of circulating platelets may take place if stored blood is used in infants receiving exchange transfusions for hemolytic disease of the newborn. Considering the small volume of blood required, the use of stored blood constitutes an unnecessary risk in this procedure.

Patients on extracorporeal circuits frequently develop thrombocytopenia because the blood used to prime the pump is usually not absolutely fresh and, even if it is, platelets are destroyed within the bypass circuit. In addition, a defect in platelet function also has been demonstrated.<sup>335</sup> In order to prevent postoperative bleeding, platelets may be given at the completion of the operation.<sup>338</sup>

**THROMBOCYTOPENIA DUE TO SPLENIC POOLING.** Massive splenomegaly is frequently associated with a moderate degree of thrombocytopenia because of the pooling of platelets within the spleen (Chapter 45). Under most circumstances, platelet levels do not fall below  $60$  to  $80 \times 10^9/l$  and no hemorrhagic tendencies develop. However, a critical decrease in platelets may occur during acute bleeding episodes and platelet transfusions then become mandatory. Because of the trapping of platelets in the spleen, a large number of platelets may have to be given in order to obtain the desired increase.<sup>294</sup> Platelet transfusions also may be required during portocaval shunt procedures; concentrated platelets should be available before the start of the operation, as they cannot usually be obtained quickly enough in an emergency.

### Qualitative Platelet Defects

Patients with *congenital abnormalities* of platelet function (Chapter 35) do not usually require platelet transfusions except to control specific bleeding episodes or to prepare for a surgical procedure. Under such circumstances, platelet transfusions effectively prevent or control hemorrhage. When the platelet defect is acquired, and especially when it is related to an abnormal plasma environment, therapy may not be as effective. Thus the acquired platelet defect that is found in uremic patients is best corrected by dialysis<sup>315</sup>; in the absence of dialysis, transfused platelets quickly acquire the same defect as that present in the patient's own platelets. When the platelet defect is due to acquired causes such as the ingestion of aspirin or other drugs interfering with platelet function (Chapter 35), the response to transfusion is much more favorable. For, while the platelet defect due to aspirin may be discernible for a week,<sup>314</sup> the intact drug is quickly hydrolyzed.<sup>314</sup> As a result, platelets given even a few hours after aspirin ingestion no longer acquire the same defect, even though free salicylate is still detectable in the circulation.

### Complications

The complications of platelet transfusion are not as common or serious as those accompanying whole blood transfusion. They include (1) febrile transfusion reactions, (2) infectious hepatitis, (3) bacterial contamination, and (4) post-transfusion purpura. *Febrile transfusion reactions* would appear to be mostly due to infusion of incompatible leukocytes contaminating platelet preparations,<sup>269</sup> since purified platelet preparations do not cause febrile reactions in sensitized recipients.<sup>278</sup> It has been suggested, however, that serotonin released from sensitized and sequestered platelets may cause flushing.<sup>269</sup> In general, patients with febrile reactions have lower post-transfusion increments in circulating platelets than do those without these reactions. The reactions are self-limited and may occur immediately or as long as 12 hours

after transfusion; they rarely last more than 24 hours. Chills are frequent and hives have been observed. The dangers of *infectious hepatitis* have been described in Chapter 11. *Bacterial contamination* may be a problem if platelets are stored at room temperature,<sup>297</sup> but this danger is minimized by using a closed collecting system and observing good technique during the collection and processing of blood. The problem of *post-transfusion purpura* has been discussed elsewhere (Chapter 34).

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# Part III

## Disorders of the Red Cells

## *The Approach to the Patient with Anemia*

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though the conditions that result in anemia encompass nearly the full spectrum of human disease, an orderly approach to the diagnosis in the patient with anemia is usually fruitful.

"Anemia" is not a diagnosis in itself, but merely an objective sign of the presence of disease. The correct diagnostic terminology for a patient with anemia requires the inclusion of the pathogenesis of the anemia (eg, iron-deficiency anemia, due to carcinoma of the colon; anemia of chronic renal disease; hemolytic anemia due to administration of alpha-methylpapa). The reason for this is simple and fundamental: the correct treatment requires an understanding of the pathogenesis of the condition (Chapter 1).

Furthermore, as discussed in Chapter 3, it is necessary to think of the circulating red cells and of the bone marrow from which they arise as a single functional entity, to which the term "erythron"<sup>22</sup> has been applied. The erythron includes the normoblasts at all stages of maturation and the reticulocytes, as well as the erythroid-committed stem cells (Chapter 2) which lack morphologic features to identify them as precursors to red cells, and, in the circulation, the mature red cells. In the bone marrow, the interstitial tissue of the erythron is made of reticulum, fine capillaries, and fat. In the circulating portion of the erythron, the plasma acts as interstitial tissue. In addition to serving as the medium of transport for the red cells from the lungs to the other tissues, plasma also is the continuing source of glucose for the met-

THE detection and diagnosis of anemia are frequently the focus of attention in the care of patients because accurate quantitation is not difficult to achieve and rational analysis of the problem is possible. Even



abolic activities of the erythrocytes (Chapter 3) and the means for removal of its catabolic products. Although plasma is normally isotonic with red cells, its high sodium and low potassium concentrations present a hostile ionic environment for which the erythrocyte must compensate by active metabolic work (page 100). When they fail to do so, hemolytic disease results (Chapter 20). Other adverse factors that may be presented by the interstitium include antibodies that react with red blood cells and may lead to destruction of the main substance of the erythron (Chapter 27).

"Atrophy" of the erythron may occur in aplastic anemia (Chapter 56), whereas hypertrophy occurs in polycythemia vera (Chapter 30). In addition, since the hematopoietic system functions as a physiologic unit, when erythropoiesis is stimulated, as a rule it is found that there is increased leukopoiesis and increased thrombopoiesis. Thus, following acute blood loss and in hemolytic anemia, there may be not only reticulocytosis but also moderate or even marked leukocytosis accompanied by a greater number of the younger forms of leukocytes than are ordinarily present ("shift to the left"). The quantity of platelets in the circulation is also likely to be increased.

The diseases afflicting the erythron may be considered in the same categories as those affecting other organ systems, viz, congenital (Chapters 21 to 26) or acquired disease (Chapters 14 to 19; 27 to 29); those due to hypoplasia or atrophy (Chapter 56), neoplasia (Chapter 46), infection (Chapter 18), trauma (Chapter 28), the action of physical (Chapter 28) or chemical (Chapter 23) substances, and so forth. Often more useful for diagnostic purposes, however, are classifications based upon the altered physiology of the erythron in disease states. These classifications are discussed later in this chapter.

## The Approach to the Diagnosis

The first step in the diagnosis of anemia is the detection of the presence of anemia per

se. This requires the accurate *measurement* of the pertinent values, as discussed in Chapter 1, and comparison of these values with suitable *reference values* for healthy individuals, given in Appendix A. The second step is the investigation of the pathogenesis of the anemia. Sufficient regard for the first step is required if one is to avoid the performance and expense of needless diagnostic studies and treatment of nonexistent anemia, as well as the converse, overlooking the presence of slight anemia and thereby neglecting an important clue to the presence of a disease that might be readily amenable to treatment.

The term "anemia," as it is generally used in clinical medicine, refers to a reduction below normal in the *concentration* of hemoglobin or red blood cells in the blood. The concentration is best measured as the VPRC rather than by enumeration of the red blood cells, because the VPRC offers greater precision and accuracy, and also greater ease of standardization and maintenance of equipment, than do other approaches. Simple but accurate methods are discussed in Chapter 3. Alternatives to the VPRC are discussed in Chapters 1 and 3. As explained there, measurement of hemoglobin is a satisfactory alternative but does not provide other useful information. Strict attention to technique and standardization is required no matter what method is applied, if *meaningful diagnoses* are to result.

It must be remembered that the mean normal value and the lower limits of the "normal" range depend upon the age (childhood or adult life) and gender of the subjects, as well as the altitude of residence. After puberty, the values for males are higher than for females. Apart from these differences, one must be wary of a laboratory that assigns normal values or ranges that differ from the established values cited here. Either inaccuracy in measurement or faulty selection of the reference population is implied, and inaccurate results of work done on patients must also be suspected.

The selection of a reference population is fraught with problems.<sup>3, 4, 123, 145</sup> Nevertheless, the physician must not equate locally

prevalent values with normal values. To do so would erroneously lead to the conclusion that in a particular subpopulation or geographic region a different, lower standard applies. This error has been made in surveys of large populations. Rather than recognize the prevalence of anemia, some authors have advised that the lower limit of normal values for women must be set below the values given previously by Wintrobe,<sup>140</sup> or subsequently by WHO<sup>145</sup> and the AMA Council on Food and Nutrition.<sup>3</sup> It has been found that 20 to 30% of some populations surveyed in the United States<sup>74</sup> and in Sweden<sup>56,61,133</sup> have iron-deficiency anemia. To have accepted such persons as normal by reducing the lower limit of the normal hemoglobin to 10.2 g/dl<sup>74</sup> or 11.5 g/dl,<sup>56</sup> in order to coincide with the observations in the survey population, is a disservice to patients and misleads physicians.<sup>115</sup> Strict adherence to the values listed is advised. Even though statistical interpretation of normal distribution curves leads us to expect that 2.5% of a healthy population might have hemoglobin or hematocrit values lower than 2 SD below the mean, the burden of proof is on the physician to show that disease is absent in every patient in whom the hemoglobin or hematocrit values fall below the limits cited in Appendix A.

Regarding anemia, it is important to have a clear concept of the distinction among three measurements: (1) the total number or volume of red blood cells in the circulation (the "red cell mass"); (2) the total quantity of blood, meaning red blood cells and plasma, in the circulation (the "blood volume"); and (3) the ratio of these two (the "body hematocrit"), which is a measure of concentration and is closely reflected by the venous blood hematocrit, red blood cell count, and hemoglobin concentration. Reduction in blood volume has been called "oligemia" or "hypovolemia," and reduction in the red cell mass has been called "oligocythemia." The distinction between a reduction in the concentration and a reduction in the total quantity of red cells is required for clinical practice, but the term "oligocythemia" is used less

often than the phrase "reduction in red cell mass" to express the latter finding. Conceptually, a reduction in the total quantity of red blood cells or hemoglobin in the circulation may be a correct definition of anemia, but it is not a practical one.

In the great majority of instances, when oligocythemia is present the total blood volume remains at nearly normal values because of compensatory expansion of the plasma volume. Consequently, as a rule, the reduction in the concentration of red blood cells very nearly reflects the reduction in the total quantity of red blood cells in the circulation. The same is true for the concentration and total quantity of hemoglobin. These facts generally obviate the need for the measurement of the blood volume to appraise the reduction in the red cell mass. The exceptions to this rule are acute blood loss, and the limited number of clinical situations that are accompanied by a significant reduction or increase in the plasma volume; these are listed in Table 13-1. An important reason that the total quantity of hemoglobin or red cells in the body is not generally used as a measure of anemia is that measurement of the blood volume requires the time-consuming and sophisticated techniques of dye or isotope dilution methods (Chapter 3). These are not suitable for broad application in large numbers of patients, nor for often-repeated determinations in a given individual.

"Spurious anemia," that is, a subnormal concentration of red blood cells due to hemodilution, as occurs in the third trimester of pregnancy,<sup>20,32,123,198</sup> is not a disorder of erythropoiesis or of the erythron and should not be treated as such. Likewise, when an increased concentration of red blood cells is found, the physician must distinguish between secondary erythrocytosis and polycythemia vera (Chapter 30) on the one hand, and hemoconcentration, due to a chronic or an acute reduction in plasma volume, on the other. In the latter, clinical signs of dehydration, hypotension in the upright posture, poor skin turgor, and high values for urine specific gravity are guiding clues. More difficult is the detection of chronic anemia in patients in

**Table 13-1. Conditions in Which There Is a Significant Disproportion between the Hematocrit and the Red Cell Mass**

- 
- A Relative increase in plasma volume** Hematocrit disproportionately low
- 1 Hydræmia of pregnancy
  - 2 Overhydration in oliguric renal failure, or congestive heart failure
  - 3 Chronic diseases and hypoalbuminemia (sometimes)
  - 4 Laennec's cirrhosis
- B Relative decrease in plasma volume** Hematocrit may be high, normal or low but is high relative to the red cell mass
- 1 Dehydration, especially saline loss
    - a Protracted diarrhea (especially in infants) cholera
    - b Intestinal malfunction (pyloric obstruction, etc.)
    - c Abdominal paracentesis with fluid restriction
    - d Peritoneal dialysis with hypotonic solutions
    - e Diabetic acidosis
    - f Extended fluid deprivation
    - g Diabetes insipidus with restricted fluid intake
  - 2 Stress erythrocytosis spurious polycythemia
- C Decrease in both plasma volume and red cell mass** Hematocrit normal, red cell mass low
- 1 Acute blood loss
  - 2 Cancer (sometimes)
  - 3 Myxedema Addison's disease panhypopituitarism
- 

whom the reduced red cell mass is masked by an associated contraction of the plasma volume.<sup>13, 17, 36, 52, 100</sup> In acute conditions, the true scope of the anemia becomes evident when hydration and homeostasis are restored. In some chronic conditions, anemia may be partly or completely masked even in the face of adequate hydration; measurement of the red cell mass or of the plasma volume is needed to appreciate the true situation.<sup>13, 17</sup> For example, hypotension on induction of general anesthesia has been attributed to hypovolemia in patients with a normal hematocrit, when the underlying disease has been carcinoma,<sup>13, 18</sup> pheochromocytoma, hypertension, or hypothermia, or when vasopressors have been administered.<sup>2</sup> Hypovolemia, perhaps as a consequence of diuretic therapy,<sup>2</sup>

also was noted in a patient with mitral stenosis and regurgitation who was scheduled for valvulotomy.

## Manifestations of Anemia

Symptoms in patients with anemia depend on five factors: (1) the reduction in the oxygen-carrying capacity of the blood, (2) the degree of change in total blood volume; (3) the rate at which (1) and (2) have developed, (4) the associated manifestations of the underlying disorder that resulted in the development of anemia, and (5) the capacity of the cardiovascular and pulmonary systems to compensate for the anemia. There is a wealth of clinical experience indicating that the hemoglobin concentration is not the sole determinant of the symptoms that may be expected, nor of the urgency with which measures must be taken to correct the anemia. Furthermore, coexistent disease, for example, coronary insufficiency or chronic obstructive bronchopulmonary disease, may become significantly symptomatic with a degree of anemia that would be well tolerated under other circumstances.

If the anemia has been insidious in onset, and cardiopulmonary disease is absent, the patient's adjustment may be so good that the hematocrit may be 0.25 l/l or less, and the hemoglobin concentration 8 g/dl or lower, before the patient has sufficient symptoms<sup>44</sup> to appreciate his true situation. It is not uncommon for patients with iron-deficiency anemia, pernicious anemia, or other types of chronic anemia to have their hemoglobin concentration fall to 6 g/dl before they are motivated to seek medical attention.<sup>45</sup> In children, particularly, there may be little apparent restriction of capacity for physical exertion, despite the presence of severe anemia.<sup>39</sup> The physiologic adjustments that take place chiefly involve the cardiovascular system and changes in the hemoglobin-oxygen dissociation curve. These will be discussed shortly.

The symptoms of acute hemorrhage, which are discussed in Chapter 19 (page 695), are chiefly related to hypovolemia, rather than to

anemia. Depending on the amount of blood lost, there may be no symptoms, mild hypotension, or progressively severe degrees of shock.<sup>135</sup>

### Acute Hemolytic Reaction ✓

When there is an acute hemolytic reaction, there may be acholuric jaundice; and, if intravascular hemolysis occurs, there may be hemoglobinemia, methemalbuminemia, and hemoglobinuria, as well as fever and abdominal or back pain. These are related to the sudden release of hemoglobin, and to its destruction and disposal (Chapter 20). They occur in addition to the cardiorespiratory symptoms that result from the anemia per se.

### Chronic Anemia

In chronic anemia, the reduction in the concentration of the oxygen-carrying pigment in the blood has a more meaningful correlation with the cardiovascular adjustments that must be made than does the magnitude of the deficit in the total quantity per se of red blood cells or of hemoglobin in the circulation. The amount of oxygen delivered to the tissues by a given volume of blood is a function of (1) the concentration of hemoglobin, (2) the percent saturation of hemoglobin with oxygen, (3) the hemoglobin-oxygen dissociation curve, and (4) the tissue oxygen tension. When fully saturated with oxygen, 1 g of hemoglobin will bind 1.34 ml of oxygen. At a normal hemoglobin concentration, 100 ml of arterial blood contains about 20 ml of oxygen, whereas the same volume of mixed venous blood contains approximately 15 ml, the difference—amounting to about 25% of the initial quantity of oxygen—being accounted for by the extraction of oxygen by the tissues. If a patient's hemoglobin concentration were only 7.5 g/dl, then only about 10 ml of oxygen could be carried per dl of arterial blood. If tissue oxygen consumption is to be maintained in anemia, and there were no adjustments in the circulation, both the percent saturation of mixed venous blood and the tissue oxygen

tension would have to fall, and the percent utilization of arterial oxygen would increase. It follows, then, that the quantity of blood that must be pumped by the heart to the tissues, per minute, would be inversely related to the blood hemoglobin concentration if the other factors were invariable. In fact, however, the hemoglobin-oxygen dissociation curve does shift in chronic anemia, so that there is a reduction in the affinity of hemoglobin for oxygen,<sup>8,73,78,101,106</sup> which is helpful, as noted below, but a reduction in the arterial oxygen saturation<sup>101,109</sup> also occurs and this is disadvantageous. The demand for a greater cardiac output is first accomplished by an accelerated heart rate, rather than by a change in stroke volume.<sup>24,50</sup> Hence, tachycardia at rest and especially upon small exertion is characteristic of the patient with moderate or greater degrees of anemia.

### 2,3-DPG

The binding and release of oxygen by hemoglobin are profoundly affected by the variations in the concentration of 2,3-diphosphoglyceric acid (2,3-DPG) which are known to occur in the red cell in disease.<sup>7,28,60</sup> (Also see Chapter 3, page 107.) The oxygen affinity of hemoglobin is reduced as the concentration of 2,3-DPG increases, and the converse is also true.<sup>62</sup> An increase in red cell 2,3-DPG is found in chronic anemia.<sup>16,34</sup> This facilitates the delivery of oxygen to the tissues by reducing the affinity of hemoglobin for oxygen at the oxygen tensions found in capillaries.<sup>98,130</sup> At alveolar oxygen tensions, the small change in oxygen affinity that is due to the increased red cell 2,3-DPG does not significantly affect the uptake of oxygen by the red cells in the lungs. The net result favors the heart by allowing the tissues to extract oxygen during exercise at a lower cardiac index for an equivalent oxygen consumption and work load than otherwise would be the case.<sup>98</sup> It has been calculated that DPG-induced changes in hemoglobin affinity for oxygen may compensate for up to half the oxygen deficit in anemia.<sup>98</sup>

## Cardiovascular Adjustments

The cardiac index generally is increased in anemia, and the arteriovenous oxygen difference is narrowed.<sup>24,116</sup> Central venous and intracardiac pressures are not altered, although at times the right atrial pressures may be elevated. The total circulating blood volume may decrease,<sup>21</sup> although this does not occur uniformly.<sup>122</sup> When the hemoglobin concentration is less than 7 g/dl, the cardiac output is nearly always increased,<sup>24</sup> and when it is less than 5 g/dl, the increase in cardiac index has been found to be due mainly to an increase in stroke volume and, to a lesser extent, in heart rate,<sup>107,108</sup> even with exercise. By contrast, in persons without anemia the increase in cardiac output accompanying exercise is mainly due to tachycardia. In children with severe anemia, the mean cardiac index was found to be approximately the same as in adults with equally severe anemia. However, in children this state was achieved by greater tachycardia and a lesser increase in stroke volume than in comparably anemic adults.<sup>29</sup> Nevertheless, the capacity of children with chronic anemia to endure exercise burdens generally is excellent.<sup>279</sup> If congestive heart failure develops, there is usually some underlying heart disease, but the latter may not be the case in adults if the hemoglobin concentration is less than 5 g/dl.<sup>12,59,107,132</sup> In sickle cell anemia, heart failure has occurred at less severe degrees of anemia.<sup>75</sup>

Cardiac compensation may be marginal in severe anemia, and congestive heart failure may be precipitated by blood transfusion<sup>59</sup> unless precautions are taken<sup>19</sup> (Chapter 11). As the cardiovascular system adapts to anemia, generally the velocity of blood flow is increased<sup>18,107,108</sup> and hence the circulation time is shortened; this condition may persist even with the development of congestive heart failure.<sup>54</sup> Studies have shown that mild or moderate anemia is accompanied by a greater coronary flow per unit of left ventricular work; ultimately, however, when the hemoglobin concentration is reduced below

half of normal, reduction of ventricular function is observed, presumably because the coronary flow has approached maximum.<sup>31</sup> Whereas reflex vasoconstriction is a feature of the response to acute blood loss,<sup>50</sup> in chronic anemia the peripheral vascular resistance is lowered, and this plays a dominant role in the high-output, hyperkinetic circulatory response.<sup>48</sup> Increasing peripheral resistance, as with methoxamine or orthostatic stress, reduces cardiac output.<sup>48</sup>

Changes in peripheral resistance are regional, rather than generalized. Muscle blood flow is increased, whereas skin blood flow is reduced.<sup>1</sup> Cerebral blood flow is increased.<sup>66</sup> Renal blood flow is diminished.<sup>33</sup> Usually the systemic and pulmonary artery systolic pressures are unaffected, but the diastolic pressures are lower.<sup>107</sup>

Cardiac enlargement in anemic patients is ascribed to dilatation. In rats made anemic by iron deficiency, however, cardiac hypertrophy occurred. The total quantity of protein, RNA, and DNA in the myocardium was increased, but their concentrations were unchanged.<sup>77</sup>

## Respiratory and Circulatory Symptoms

Many times, respiratory and circulatory symptoms are only noticeable following exertion or excitement; however, when the anemia is sufficiently severe, dyspnea and awareness of vigorous or rapid heart action may be noted even at rest.<sup>24</sup> A humming or whirring sound in the head, which is attributed to the rapid blood flow through cranial arteries, may be bothersome to the patient and signal the point at which significant anemia has developed. The rapidity of the onset of anemia, its severity, the age of the patient, and the capacity of the cardiovascular system to adjust to it govern the clinical presentation. When anemia develops rapidly, shortness of breath, tachycardia, pallor, "dizziness" or faintness, particularly upon arising from a sitting or recumbent posture, and extreme fatigue are prominent. In chronic anemia, only moderate dyspnea or palpitation may

anemias due to the deficiency of specific substances and can be treated successfully.<sup>6</sup> When achlorhydria is present, it is common for the patient to have a stool in the morning, immediately on arising, and one or two later in the day.<sup>7</sup> Bouts of diarrhea are not uncommonly misconstrued as "intestinal flu." Change in stool habits may be an important lead to neoplasms of the colon and rectum underlying the anemia.<sup>8</sup> The possibility of blood loss must be considered. The significance of tarry stools is often not appreciated by patients, and specific inquiry is necessary. The amount of blood lost from hemorrhoids may be overlooked by patients, or overestimated. In men, occult blood loss is most often from the gastrointestinal tract. In women, in addition to the preceding, it is necessary to appraise the actual amount of blood lost during menstruation, although this is notoriously inaccurate if only a routine inquiry is made. The number of pregnancies and abortions, and the interval since the most recent of these, is also important, for each represents significant iron loss. In a child or adolescent, the rate of growth is noteworthy.

<sup>1</sup> The dietary history is quite important but it is difficult to obtain an accurate one. It is obviously not sufficient to learn that the patient eats certain foods. It is important to know how much or how little of these foods is consumed, and to remember that the patient may deliberately deceive the physician because of embarrassment over eating habits or financial restrictions. The patient must be questioned regarding the diet, meal by meal, and quantitative information should be obtained if at all possible. A record regarding dietary idiosyncrasies and cooking habits may be valuable, especially with reference to folic acid intake.<sup>2</sup> Changes in weight are most important.

<sup>3</sup> The presence or absence of fever must be known, for if present it suggests infection, lymphoma or other neoplasm, or collagen disease.<sup>4</sup> Inquiry regarding cough, pleurisy, and hemoptysis is important since either infection or neoplasm may be causes of the anemia.<sup>5</sup> Pains in the limbs, paresthesias, and difficulty in walking suggest pernicious ane-

mia.<sup>6</sup> Abnormal color of the urine, suggesting blood or hemoglobin, may signify urinary tract disease or hematologic problems. Bilirubin does not occur in the urine in uncomplicated hemolytic anemia, but a darker than normal color may result from the increased excretion of urobilinogen and its conversion to urobilin.

<sup>7</sup> Bruises, ecchymoses, and petechiae are other important points in the history. Their presence indicates that the disorder producing the anemia is not confined to the erythron, and that an additional disturbance of the other marrow elements or of the liver must be sought. Alternatively, the anemia itself may be the consequence of blood loss resulting from a disorder of hemostasis.

In all instances, the presence or absence of symptoms suggesting an underlying disease such as chronic renal disease, chronic infection, an endocrinopathy, or malignancy must be explored.

The physical examination should include a careful evaluation of the optic fundi and the nervous system. Scleral icterus should not be overlooked, and it should be recalled that the yellowish cast of incandescent lights will obscure it. Careful palpation for sternal tenderness should not be omitted. In acute leukemia (Chapter 47), rather frequently one will find a small, acutely tender area near the mid or lower third of the sternum of which the patient may have been quite unaware. This can be easily overlooked unless it is particularly sought out, as it is frequently present even in the absence of tenderness of the bones of the extremities. A systematic check for palpable enlargement of the lymph nodes must be made, with reference to infection, lymphoma, leukemia, and metastatic carcinoma (Chapter 40).<sup>5</sup> The heart cannot be ignored, for murmurs may yield the first evidence of subacute bacterial endocarditis, thereby explaining the presence of anemia. In the abdominal examination, in addition to palpation of the liver and spleen, the kidneys should be given attention. Physical examination may reveal the first clue to renal cell carcinoma as the cause of obscure anemia. Neither the pelvic nor the rectal examination can be ne-

glected, for tumor or infection in these regions may also cause anemia.

Even when the color of the *urine* does not suggest blood, the routine urine analysis should include a test for occult blood. This simple, confirmatory procedure should also be performed when hemoglobinuria is suspected, or apparent. However, a positive reaction may also be due to hematuria or even myoglobinuria. *Hematuria* may be differentiated from the other conditions by finding red blood cells upon microscopic examination of the urine, or by centrifuging a fresh urine specimen and thereby clearing the bloody color from the supernatant portion and depositing the red blood cells in the bottom of the tube. Hematuria may be the result of disease of the kidneys or urinary tracts themselves, or the consequence of thrombocytopenia or of acquired or congenital abnormalities of the blood coagulation system. The sickle cell trait may be accompanied by innocuous hematuria.

The finding of hemoglobinuria implies intravascular hemolysis, which is usually a very serious situation. One must be careful not to confuse true hemoglobinuria with the release of hemoglobin into the urine from lysis of red cells in a specimen that has stood a while prior to examination. The circumstances surrounding routine urine analyses by large laboratories may obscure this important distinction, unless the physician or the clinical pathologist personally intervene.

## Morphologic Features in Anemia

So much information can be obtained from the examination of the stained blood film that it is always worthwhile for the trained physician to examine it himself. In clinical practice, reliance is too often placed on technicians for blood examinations. Whereas, under ordinary circumstances, most of the quantitative determinations are too time-consuming to be done by the physician, microscopic examination of the blood film can be made in a short time with great benefit resulting. This also serves as a check on the blood counts. Our approach for evaluating the

blood film is detailed in Chapter 1. The value of the VPRC as a simple, quick, and accurate aid to the physician has been discussed (page 115). Together, the blood film and the VPRC, including inspection of the hematocrit tube to determine the icterus index and the volume of packed white cells and platelets (VPW & PI) (Frontispiece), may allow solution of the diagnostic problem.

The forms and structure of the red blood cell are evaluated and compared with the red cell indices; polychromasia of erythrocytes is noted if present; and any abnormalities of the leukocytes or platelets are observed (page 27). Such an examination quickly and easily identifies cases of *macrocytic anemia* (Chapter 14), *microcytic-hypochromic anemia* (Chapter 16) (Table 13-3), and the spherocytosis and polychromasia indicative of *hemolytic anemia* (Chapter 20). The finding of *oval macrocytes*, particularly when accompanied by neutropenia, hypersegmented neutrophils, relative lymphocytosis, and sometimes thrombocytopenia, suggests *megaloblastic anemia* (Chapter 14). Marked *polychromatophilia* of the red blood cells implies a high reticulocyte index, hence accelerated red cell production. When erythropoiesis is stimulated, as, for example, following *acute blood loss*, there may be not only *reticulocytosis* but also moderate or even marked *thrombocytosis* and *leukocytosis* accompanied by an increase in the younger forms of leukocytes not ordinarily seen in such numbers in the blood ("shift to the left").

Other strong clues regarding the cause of the anemia can be adduced from the examination of the blood film (Table 13-3). The presence of *helmet cells* and other *schistocytes* implies a microangiopathic or traumatic type of *hemolytic anemia* (Chapter 28), and should also suggest the possibilities of *uremia*<sup>45</sup> and *malignant hypertension*.<sup>28</sup> *Target cells* suggest the presence of the abnormal hemoglobins C<sup>129</sup> and E<sup>33</sup> (Chapter 24), *thalassemia* (Chapter 26), *liver disease with jaundice*,<sup>38</sup> or the post-splenectomy state<sup>40,119</sup>; they also are seen in obstructive jaundice.<sup>38</sup> *Leptocytes* (Gr. *leptos*, fine) are thin erythrocytes, usually of normal or slightly greater than normal diameter, which occur in heterozygotes for thalas-

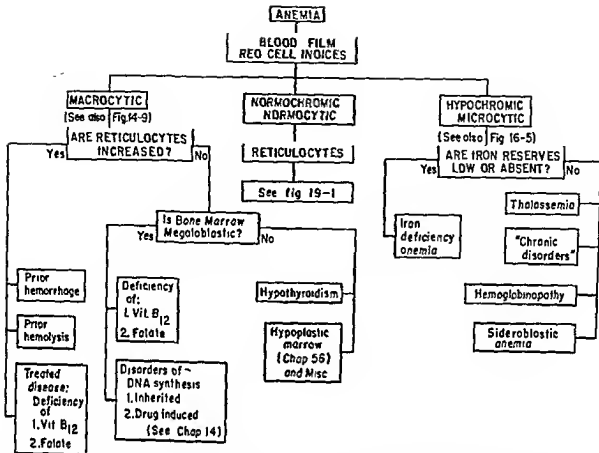


Fig 13-1. Diagram to illustrate successive steps in the differential diagnosis of anemia beginning with the examination of the blood smear and the measurement of red cell indices. The history and physical examination may make it unnecessary to carry out the subsequent steps, namely, reticulocyte index, bone marrow examination, and evaluation of iron reserves as judged by serum iron and total iron-binding capacity and staining of bone marrow for iron.

semia even when target cells are not evident (Chapter 26). The discovery of normocytic-hypochromic erythrocytes on microscopy, in the face of microcytic-normochromic red cell indices, is the hallmark of leptocytosis. This combination of findings, especially when accompanied by basophilic stippling of the red cells, suggests heterozygosity for a thalassemia gene (Chapter 26). The finding of a mixture of hypochromic and normochromic erythrocytes in an anemic patient who had not been transfused or treated with iron ("dimorphic anemia") suggests a sideroblastic anemia (Chapter 18). Elliptocytes, wherever they occur,<sup>11,70</sup> are a readily recognized indication of an anomaly of the red cell that may be accompanied by hemolytic disease (Chapter 21).<sup>11</sup> They may also be found in thalassemia and other conditions (Chapter 20).

A variety of red cells with one or more spiny projections has been described ("spicule cells") and of these two types can be distinguished, acanthocytes and echinocytes. Acanthocytes are red cells with several irregularly spaced, large and coarse projections on their surface, which vary in width and length.<sup>23</sup> They may resemble spherocytes with pseudopods (Chapter 20) and typically are seen in a rare syndrome characterized by steatorrhea, retinal degeneration, mild hemolytic anemia, and deficiency of betalipoprotein (Chapter 21).<sup>94</sup> Similar cells (spur cells) are described in a severe hemolytic syndrome accompanying Laennec's cirrhosis.<sup>37,70,118,121</sup> (Chapter 19) and have been seen in association with hemolytic anemia in metastatic liver disease.<sup>72a</sup> Such cells may also be found in fresh, wet preparations. In contrast, in echinocytes



Table 13-3. Description and Significance of Various Forms of Red Corpuscles

Type of Cell	Description	Physiologic Significance	Clinical Disorders
Macrocyte	Larger than normal ( $> 8.5 \mu\text{m}$ diameter) Well filled with hemoglobin	1 Young RBC (skipped generation early loss of nucleus) 2 DNA synthesis impaired megaloblastic maturation	1 Accelerated erythropoiesis 2 $B_{12}$ or folate deficiency
"Thin" macrocyte	Diameter increased but MCV normal, often hypochromic (see target cell)	Membrane cholesterol and lecithin increased	Liver disease post splenectomy
Hypochromic cell	Exaggeration of normal central pallor usually also microcytic	Failure of hemoglobin synthesis due to 1 lack of iron 2 defective globin synthesis 3 defective porphyrin synthesis	1 Iron deficiency anemia anemia of chronic disease (?) 2 Thalassemia, some hemoglobinopathies (C, E) 3 Sideroblastic anemias
Target cell	Hypochromic, with central pigment, thin cell surface/volume ratio increased	1 Splenectomy decreases rate & extent of loss of lipids from reticulocytes 2 Accumulation of both cholesterol and phospholipid on RBC 3 Congenital	As for hypochromic cells; also 1 post splenectomy 2 in liver disease, especially obstructive jaundice 3, LCAT deficiency Thalassemia
Leptocyte	Thin, hypochromic cell, diameter normal, MCV decreased		
Microcyte	Smaller than normal ( $< 7.0 \mu\text{m}$ )	Differs according to whether or not it is 1 well filled with hemoglobin 2 shape is normal	See below
Spherocyte	Spherical, not hypochromic, usually also microcytic, surface/volume ratio decreased	1 RBC membrane abnormality 2. RBCs lose fragments after impact with fibrin strands, walls of diseased vessels, and artificial surfaces in the circulation	1 Hereditary spherocytosis 2. Acquired immunohemolytic anemia

<i>Elliptocyte</i>	Elliptical in shape, not hypochromic	1 Hereditary abnormality 2 Acquired alteration	1 Hereditary elliptocytosis 2 In various anemias, especially megaloblastic
<i>Sickle cell</i>	In shape of sickle, form assumed especially on deprivation of oxygen	Molecular aggregation of Hb S	Hb S trait or disease Also seen with Hb I, Hb C <sub>Capetown</sub> , Hb C <sub>Philadelphia</sub>
<i>Schistocyte</i>	Helmet- or triangular-shaped, fragmented or greatly distorted RBC, smaller than normal	RBC lose fragments after impact with fibrin strands, walls of diseased vessels, and artificial surfaces in the circulation	1 Microangiopathic hemolytic anemia 2 Hemolytic anemia due to physical agents 3 Also in uremia, malignant hypertension
"Teardrop" RBC	Shape of drop, usually microcytic often also hypochromic	Distorted or fragmented RBC	1 Especially in myelofibrosis 2 Less frequently in other forms of anemia; eg thalassemia
<i>Spicule cell</i> may be (a) <i>Acanthocyte</i> ( "spur cell" )	RBC with spiny projections on surface Has 5 to 10 spicules of various lengths irregular in spacing and thickness	Differs according to type (a) Ratio of cholesterol/lecithin of RBC membrane increased when associated with liver disease Can be converted to normal shape by non-ionic detergents	1 In abetalipoproteinemia 2 Liver disease with hemolytic anemia 3 Post splenectomy (few) 4 Pyruvate kinase deficiency
(b) <i>Echinocyte</i> (Sea urchin cell crenated cell, burr cell)	Has 10 to 30 spicules, evenly distributed over surface of RBC	(b) Result of alteration of intra- and extra-cellular environment Can be brought about by accumulation of fatty acid or lyssolecithin on RBC surface or both, as result of changes in plasma or in RBC metabolism	1 Uremia, bleeding peptic ulcer, Ca stomet 2 Commonly artifactual 3 Pyruvate kinase deficiency
<i>Pyknotocyte</i>	Distorted and contracted RBC, similar to echinocyte		"Infantile pyknotocytosis"
<i>Stomatocyte</i>	Uniconcave, as contrasted with normal biconcave RBC, slit-like instead of central pallor in RBC	1 Hereditary Primary defects in membrane structure or function resulting in abnormalities of cation permeability, content and flux 2 Acquired alteration in cation content and flux	1 Hereditary stomatocytosis, several forms 2 Smaller numbers seen in alcoholic cirrhosis, acute alcoholism, obstructive liver disease, malignancies, etc, and perhaps as artifact

("sea urchin" cells) or burr cells the spicules are regularly spaced and more uniform in size and more numerous than the projections of acanthocytes. Echinocytes essentially resemble artificially produced crenated cells. They have been described in uremia, carcinoma of the stomach, and in acute blood loss.<sup>114</sup> There are conflicting views regarding their origin. The original report considered them to be true poikilocytes because they could be seen in wet preparations.<sup>114</sup> However, in many instances they may well be true crenated red cells formed during the preparation of the blood film.<sup>41</sup> It has also been proposed that they result from rupture of marginal, intra-erythrocytic vacuoles.<sup>15</sup> The finding of spicule distortions of erythrocytes also should suggest the possibility of the pyruvate kinase type of hemolytic anemia,<sup>96</sup> although spicules are not unique to this disorder.<sup>92</sup>

Stomatocytes are erythrocytes with a well-demarcated silt-like appearance instead of the usual circular zone of central pallor (Fig. 13-2).<sup>82,90</sup> Hemolytic anemia of severe<sup>82</sup> or mild<sup>91</sup> form accompanies the hereditary anomaly. Stomatocytes are not as uncommon as once thought<sup>47 83,96</sup> (Chapter 20).

Sickle cells characterize the disease due to the presence of the anomalous hemoglobin S in the erythrocytes (Chapter 25). When sickle cells are present on the ordinary blood film the diagnosis of sickle cell anemia, or one of the closely related sickle-hemoglobin syndromes, is strongly suggested. However, sickle-like cells can also be produced in the absence of hemoglobin S (page 845). Intra-erythrocytic crystals suggest the hemoglobin SC syndrome (Chapter 25).

Teardrop-shaped poikilocytes suggest myelofibrosis or other myelophthitic processes, especially when normoblasts and a few immature granulocytes are also seen on the blood film (Chapter 57). Howell-Jolly bodies are most commonly found after splenectomy, but may also be an indication of splenic atrophy following multiple infarctions (page 832), hemolytic anemia, or megaloblastic anemia (page 569). Cabot rings also are seen in these disorders.

If any of the above are detected, the diagnostic possibilities regarding the anemia are sharply narrowed. For further steps in the evaluation and treatment of the indicated condition, one should follow the procedures discussed in the corresponding chapters of this book.

### Roentgenography (X-ray)

When the history, physical examination, and initial evaluation of the blood and urine have not led to the recognition of an underlying disease process that explains the anemia, the examination should be supplemented by roentgenography. A roentgenographic film of the chest may suggest pulmonary tuberculosis or other infection, or unsuspected mediastinal enlargement due to lymphoma or other malignant disease. Roentgenograms of the bones may lead to the discovery of osteolytic lesions due to multiple myeloma or metastatic carcinoma; or of tumors, periosteal elevation suggesting leukemia, or even osteosclerosis.

### Radioisotope and Erythrokinetic Studies

The use of radioisotopes and the study of erythrokinetics (Chapter 4) are unnecessary for diagnosis in the great majority of cases of anemia, although they have been important in helping to elucidate the pathogenesis of many forms of anemia and in arriving at a diagnosis in some cases in clinical practice. These and other procedures involving special laboratory equipment and techniques, which previously were done only in certain large medical centers, are now available at many hospitals throughout the world. However, correct operation of the equipment and conduct of the technical aspects does not assure a useful result. The interpretation of the data in disease is even more difficult than in the normal state, because many of the assumptions upon which the normal model was created need not apply equally in different disease states. Changes in the red cell mass, or the rate of erythropoiesis during an erythro-

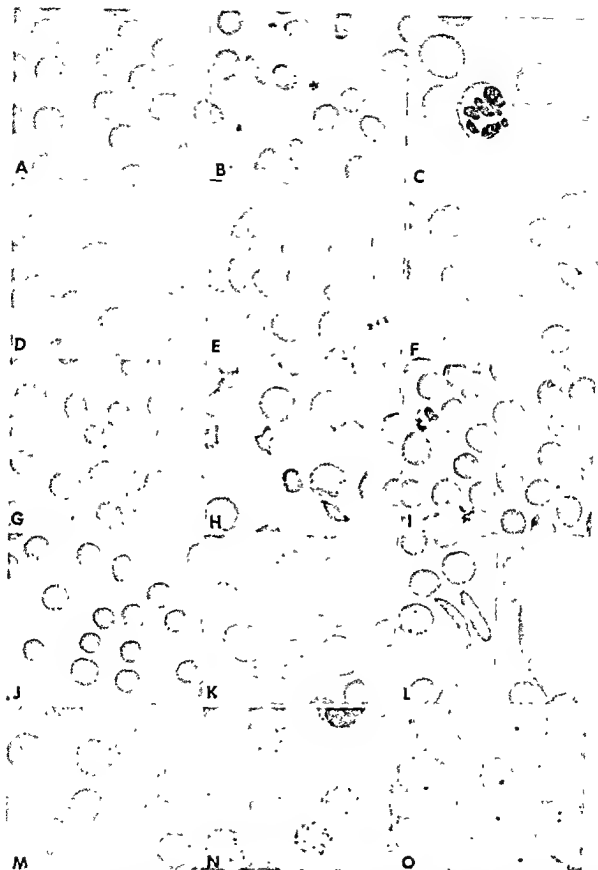


Fig. 13-2. Appearance of red corpuscles in various disorders. A Normal blood smear. B Hypochromic microcytic anemia (iron deficiency). C Macrocytic anemia (pernicious anemia). D Macrocytic anemia in pregnancy. E Hereditary elliptocytosis. F Myelofibrosis. Note "tear-drop" corpuscle. G Hemolytic anemia associated with prosthetic heart valve. H Microangiopathic anemia. I Stomatocytes. J Spherocytes (hereditary spherocytosis). K Sideroblastic anemia. Note the double population of red corpuscles. L Sickle cell anemia. M Target cells (post-splenectomy). N Basophilic stippling in a case of unexplained anemia. O Howell-Jolly bodies (post-splenectomy).

kinetic study or during measurement of red cell survival with radioisotopes, may invalidate interpretations based on comparison with normal values established in the "steady-state."

When the clinical evaluation with the accompanying simple blood, urine, and roentgenographic studies does not permit identification of the cause of the anemia, it is best to proceed with an evaluation of the mechanisms of red cell production and destruction.

## Classification of Anemia

### Pathogenetic Classification

Broad pathogenetic groups are the basis of one system of classification (Table 13-4). Thus, anemia may be attributed to (1) loss of blood, (2) excessive destruction of mature red cells, and (3) impaired red cell production. This system of classification implies a preliminary analysis and classification of data in order to place the case into one of the three main groups and one of several principal subgroups. Certain conditions, however, can be classed equally well in two or more locations; eg, although pernicious anemia is the result of a conditioned nutritional deficiency (Chapter 15), it is associated with signs of accelerated red cell destruction.

### Morphologic Classification

Morphologic classification of anemia (Table 13-5) was the natural result<sup>141</sup> of the advances made in the study of the blood through laboratory observations. It has proven to be of practical clinical value. Characteristic changes in the size and the hemoglobin content of the red corpuscles occur in some of the various types of anemia that have been listed in the pathogenetic classification. For that reason, classification on morphologic grounds is helpful in differentiating these anemias, and treatment may be guided to some extent by such observations, as will be shown later in this chapter.

Underlying the morphologic classification is the observation that when anemia develops

**Table 13-4. Pathogenetic Classification of Anemia**

- I Blood loss**
  - A Anemia after recent hemorrhage (acute)
  - B Anemia after persistent hemorrhage (chronic)
- II Excessive destruction of erythrocytes (hemolytic disease)**
  - A Extracorpuscular factors
    - 1 Antibodies
    - 2 Infection (malaria, etc.)
    - 3 Splenic sequestration and destruction
    - 4 Associated disease states, eg lymphoma
    - 5 Drugs, chemicals, and physical agents
    - 6 Trauma to RBC
  - B Intracorpuscular defects
    - 1 Hereditary
      - a Disorders of glycolysis
      - b Faulty synthesis or maintenance of reduced glutathione
      - c Qualitative or quantitative abnormalities in synthesis of globin
      - d Abnormalities of RBC membrane
      - e Erythropoietic porphyria
    - 2 Acquired
      - a Paroxysmal nocturnal hemoglobinuria
      - b Lead poisoning
- III Inadequate production of mature erythrocytes**
  - A Deficiency of essential substances
    - 1 Iron, folic acid, vitamin B<sub>12</sub>
    - 2 Protein
    - 3 Possibly ascorbic acid
    - 4 Experimentally: copper, cobalt, pyridoxine, niacin, riboflavin, possibly pantothenic acid, thiamin
  - B Deficiency of erythroblasts
    - 1 Atrophy of bone marrow: aplastic anemia
      - a Chemical or physical agents
      - b Hereditary
      - c Idiopathic
    - 2 Isolated erythroblastopenia ("pure red cell aplasia")
      - a Thymoma
      - b Chemical
      - c Antibodies
  - C Infiltration of bone marrow
    - 1 Leukemia, lymphoma
    - 2 Multiple myeloma
    - 3 Carcinoma, sarcoma
    - 4 Myelofibrosis
  - D Endocrine abnormality
    - 1 Myxedema
    - 2 Addisonian adrenal insufficiency
    - 3 Pituitary insufficiency
    - 4 Sometimes, hyperthyroidism
  - E Chronic renal disease
  - F Chronic inflammatory diseases
    - 1 Infections
    - 2 Noninfectious diseases, including granulomatous and collagen diseases
  - G Cirrhosis of liver

Table 13-5. Morphologic Classification of Anemia

Morphologic Type of Anemia	Underlying Abnormality	Clinical Syndromes	Treatment
<b>I. Macrocytic (MCV &gt; 94, MCHC &gt; 31) <i>glu</i></b>			
<b>A Megaloblastic</b> (for details see Table 14-3)	1 Vitamin B <sub>12</sub> deficiency	Pernicious anemia, etc	Vitamin B <sub>12</sub>
	2 Folic acid deficiency	Nutritional megaloblastic anemias, sprue & other malabsorption syndromes, etc	Folic acid
<b>B Non-megaloblastic</b> (see Table 14-1)	3. Inherited disorders of DNA synthesis	Orotic aciduria, etc	According to nature of disorder
	4 Drug-induced disorders of DNA synthesis	Chemotherapeutic agents	Stop offending drug
	1 Accelerated erythropoiesis	Anticonvulsants, oral contraceptives	Folic acid
	2 Increased membrane surface area	Hemolytic anemia Response to hemorrhage Hepatic disease, obstructive jaundice, post splenectomy	Treatment of underlying disease
	3 Obscure	Myxedema Hypo- & aplastic anemia etc	
<b>II Hypochromic-microcytic (MCV &lt; 80, MCHC &lt; 31)</b>			
	1 Iron deficiency, (for details see Table 17-3)	Chronic loss of blood, inadequate diet, impaired absorption, increased demands etc	Ferrous sulfate & correction of underlying cause
	2 Disorders of globin synthesis	Thalassemia alone or with a hemoglobinopathy (Table 28-1 and 26-3)	Nonspecific
	3 Disorders of porphyrin & heme synthesis	Pyridoxine-responsive anemia, etc (Table 18-2)	Pyridoxine
	4 Other disorders of iron metabolism (Table 16-2)	See Table 16-2	
<b>III Normochromic-normocytic (MCV 82-92, MCHC &gt; 30) (See Table 19-1)</b>			
	1 Recent blood loss	Various	Transfusion, iron Correct underlying condition
	2 Overexpansion of plasma volume	Pregnancy Overhydration	Restore homeostasis
	3 Hemolytic diseases (Table 20-3)	See Table 20-3	According to nature of disorder
	4 Hypoplastic bone marrow (See Table 56-1)	Aplastic anemia Pure red cell aplasia	Transfusions, stop drug Androgens
	5 Infiltrated bone marrow	Leukemia, multiple myeloma, myelofibrosis, etc	Chemotherapy, etc
	6 Endocrine abnormality	Hypothyroidism adrenal insufficiency, etc	Treatment of underlying disease
	7 Chronic disorders	See Table 18-1	Treatment of underlying disease
	8 Renal disease	Renal disease	Treatment of underlying disease
	9 Liver disease	Cirrhosis	Treatment of underlying disease

MCV, mean corpuscular volume, in fl; MCHC, mean corpuscular hemoglobin concentration, in g hemoglobin/dl.  
 > greater than; < less than

there is not always a proportionate decrease in the number of red corpuscles, the quantity of hemoglobin, and the volume of packed red cells per unit volume of blood. When the average size or the average hemoglobin content of the red cells is altered, disproportionate changes in the measures of anemia occur. These are detected by computing the *red cell indices* (Chapter 3) and confirmed by the direct observation of the red cells on the *stained blood film* (Chapter 1). Characteristically, certain diseases cause a greater decrease in the number of red cells than in the hemoglobin or red cell mass. This reflects the fact that in those conditions the majority of the red cells produced are larger than normal, a situation that is termed "*macrocytic anemia*" and is characterized by a high MCV (page 566). In other diseases, the converse may occur; namely, there may be a proportionately greater decrease in the quantity of hemoglobin and in the volume of the red cell mass than in the total quantity of red cells. In such cases, the majority of red cells are smaller than normal; this is termed "*microcytic anemia*" and is characterized by a low MCV (page 621). In such cases, in addition to alterations in the size of the red cells, there usually is a proportionately greater reduction in the total quantity of hemoglobin than in the total quantity of red cells. This reflects a reduction in the *concentration of hemoglobin within the individual red cells*; it is termed "*hypochromic anemia*" and is characterized by a low MCHC (page 621). Finally, anemias in which, on the average, the red cells are not altered in size are called "*normocytic anemias*," and, if the MCHC is also normal, they are termed "*normochromic-normocytic anemias*."

If one first classifies anemia on morphologic grounds as described above, and then lists some of the etiologic factors that may be grouped under these categories (Table 13-5), it becomes apparent that this approach is very helpful in limiting the diagnostic possibilities when either macrocytic or microcytic-hypochromic anemia is discovered. However, it is less useful if the anemia proves to be of the normocytic type, because of the many and diverse diseases included in this

subgroup. In the latter circumstance, *kinetic considerations* regarding red cell production and destruction are very helpful in arriving at an etiologic diagnosis.

### Erythrokinetic Classification

The number of erythrocytes present in the circulation at a given time is the result of a *dynamic equilibrium* between the *production* and *delivery* of red cells into the circulation on the one hand, and their *destruction* or *loss* from the circulation on the other. Anemia can be construed to be primarily due to an alteration either in production or in destruction, or in both of these factors. From this simple concept, it is possible to develop an understanding of anemia based on the mechanism of its development. The basic data required for such an analysis of anemia are listed in Table 13-2.

The normal homeostatic mechanisms of the body bring about recovery from anemia by accelerating erythropoiesis. In an otherwise healthy individual who sustains acute anemia, whether as a volunteer blood donor or by hemorrhage as a result of trauma or disease, one finds an increase in the daily rate of production and release of new red cells into the circulation. This results in a reticulocytosis, the magnitude of which is related to the degree of the anemia. The reticulocyte count gradually returns to normal values as the normal red cell mass is restored. If anemia is persistent, then one of three explanations will obtain: erythropoiesis may be diminished or even virtually absent (*insufficient erythropoiesis*); or, even if erythropoiesis is accelerated to maximum values, it may be inadequate to compensate for continued loss of red cells by either hemorrhage (*continued bleeding*) or destruction of mature red cells within the body (*uncompensated hemolytic disease*); or, erythropoiesis may be stimulated but *ineffective* insofar as delivery of mature erythrocytes to the circulation is concerned.

The first questions to be answered in determining the kinetic basis for a given case of anemia are: "*Is erythropoiesis accelerated?*" and "*Is it effective?*" If the answers are, "Yes," then either the hematocrit should be rising

and the patient recovering or, if the anemia persists despite the delivery of greater than normal numbers of red cells to the circulation daily, there must be either persistent blood loss or active hemolytic disease.

Often hemolytic anemia is suspected because there are signs of increased bilirubin production. The plasma may be distinctly icteric, and the van den Bergh reaction reveals an increase in unconjugated ("indirect") bilirubin. Examination of the urine in such cases reveals it to be free of bilirubin, but an increased quantity of urobilinogen is present. It is useful, where the facilities exist, to measure the quantity of urobilinogen excreted in the stool as well. As a rule, in *acute* and in many instances of *chronic hemolytic anemia*, in addition to the pigmentary evidence just mentioned, increased blood destruction is also accompanied by reticulocytosis, leukocytosis, and thrombocytosis (Chapter 20). When exceptions to this rule are found, one should consider the diagnoses: paroxysmal nocturnal hemoglobinuria (Chapter 29), disseminated lupus erythematosus or other collagen disease, and lymphoma (Chapters 51 and 52).

The reticulocyte count or index (Chapter 20) provides the most simple and practical estimate of effective red cell production. If the reticulocytes are increased and serial measurements show that the patient is recovering from the anemia, then one of three mechanisms may have been responsible for the anemia: (1) a self-limited or discrete episode of hemorrhage; usually the history allows a decision in this regard, although sometimes melena may have occurred and the patient has not appreciated its significance; (2) a self-limited or discrete episode of hemolysis that has terminated with the removal of a chemical or physical agent, or the end of a transient, associated disease process that induced it (Chapter 20); or (3) a treated nutritional deficiency, (eg, of iron, vitamin B<sub>12</sub>, folic acid), in which treatment had been administered to the patient prior to the current examination, with or without a precise diagnosis.

When the reticulocytes are normal or low, erythropoiesis is presumed to be impaired.

Two general varieties of impaired erythropoiesis must be considered, namely, *insufficient erythropoiesis* and *ineffective erythropoiesis*.

Insufficient erythropoiesis is associated with a quantitative lack of erythroid precursors. In extrauterine life in man, erythropoiesis normally occurs exclusively within the bone marrow. Absence of the normal cellular constituents of the bone marrow, and their replacement by fat, results in the clinical syndrome of *aplastic anemia* (Chapter 56). If only the erythroid cell line is affected, the term "pure red cell aplasia" is used (Chapter 56). Infiltration and replacement of the bone marrow by leukoblasts, myeloma cells, myeloid tissue (chronic myelocytic leukemia), lymphocytes (chronic lymphocytic leukemia, lymphosarcoma), neoplastic cells, granulomas (tuberculosis, histoplasmosis, sarcoidosis), or fibrous tissue (myelofibrosis) are also associated with anemia (Table 13-4). It has been assumed that the infiltration has the same untoward effect on erythropoiesis as does atrophy of the bone marrow, but this assumption has never really been proved. In both of these instances the reticulocyte count tends to be reduced or normal. When all of the marrow precursors are affected, pancytopenia may result.

The diagnostic clues to infiltration of the bone marrow are: (1) normoblasts together with immature granulocytes in the circulation, (2) teardrop and other odd-shaped poikilocytes, and (3) neutropenia or thrombocytopenia, or bizarre platelets in the circulation.

Both hypoplasia and infiltration can be detected by examination of the *bone marrow*, first by aspiration, and, if that procedure yields only a hypocellular specimen, by *biopsy* (Chapter 2). Such studies reveal the cytologic and histopathologic characteristics, respectively, in the small region that had been selected for study. An infiltrative process may be demonstrated or the marrow may be found to be hypoplastic. If the marrow is normally cellular or nearly so, and does not have abnormal elements but erythropoiesis is reduced, the underlying cause may be renal



disease, endocrinopathy (Chapter 19), or certain chronic disorders (Chapter 18); or, sparsely distributed infiltrative elements, such as tumor cells or granulomas, may have been missed.

Ferrokinetic studies can serve as a functional test of the erythroid marrow as a whole (Chapter 4). For example, reduced plasma iron transport (PIT), combined with a reduced erythrocyte iron turnover (EIT), can establish that functional erythroblasts are deficient in the marrow considered as a totality. Although rarely essential, such studies complement those made on the biopsy sample of marrow and thereby reduce the likelihood of error due to an inadequate sample. Other patterns of ferrokinetic measurements of diagnostic value can also be recognized.<sup>53</sup> These can be used to classify anemia in a patient, and thereby point to the underlying disease, when other measures fail. For example, hemolytic anemia, myeloid metaplasia, chronic infection, and similar disorders have characteristic patterns on erythrokinetic measurement (Chapter 4).

The term "ineffective erythropoiesis" is used to refer to the production of erythrocytes that are so defective that they are destroyed before they leave the marrow or very shortly thereafter. Some degree of erythropoiesis is ineffective even in normal subjects (Chapters 3 and 5); however, in certain conditions, especially megaloblastic anemias, thalassemia, and sideroblastic anemias, ineffective erythropoiesis becomes greatly exaggerated. Under these circumstances, (1) the *effective* production rate appears to be considerably less than the *total* production rate, and/or (2) the rate of heme catabolism greatly exceeds that which can be accounted for by the rate of destruction of circulating red cells. *Total* production is assessed by evaluating the erythroid cellularity of the bone marrow or by measuring the plasma iron transport rate (Chapter 4, page 164). Measures of *effective* production include the reticulocyte count or index (Chapter 20) and the erythrocyte iron turnover rate (Chapter 4, page 165). When greatly increased, the excessive heme catabolism accompanying ineffective erythropoiesis

can result in an increased unconjugated bilirubin level in the plasma. Lesser degrees can be detected by measuring endogenous carbon monoxide production or fecal urobilinogen excretion (Chapter 20). These measures will be found to exceed the theoretical values for heme breakdown calculated from a red cell survival study (Chapter 5). The bilirubin produced by ineffective erythropoiesis, unlike that derived from circulating cells, acquires an "early label" from isotopic glycine (Chapter 5).

## Treatment of Anemia

The optimum treatment of anemia requires the eradication of its cause or, if that is not possible, at least modification of the underlying disorder. This necessitates the correct diagnosis, as already discussed. The general measures are purely accessory to this objective.

### Blood Transfusion

Transfusion of blood is a valuable temporary measure for the treatment of chronic anemia if the cardiovascular compensation is inadequate, or if the magnitude of the anemia is such that cardiovascular collapse is threatened. The indications for blood transfusion are discussed in Chapter 11 (page 475). The risks involved also need to be considered. Needless to say, if there is acute or chronic blood loss, the bleeding should be arrested, and volume replacement made when acute blood loss exceeds 15% of the estimated blood volume.

### General Measures

When anemia results from infections, *treatment of the infection*, whether requiring surgical or antibiotic therapy, is the correct treatment for the anemia. The administration of iron, folic acid, and vitamin B<sub>12</sub> is of no value in such cases except when there is a concurrent deficiency of one of these substances. In subacute bacterial endocarditis, military tuberculosis, or osteomyelitis, pa-

its may present with marked anemia and presence of the infection may be difficult to establish, its very existence being doubted if it is finally proven after diligent search. In these instances, and in other cases of anemia due to infection or associated with other chronic disorders, the treatment of the underlying disease is the correct treatment for the anemia.

In acquired hemolytic anemia due to drugs, in aplastic anemia, one of the most important actions that can be taken is the removal of the patient from exposure to the offending agent. It should not be forgotten that malarial infection produces acute anemia that is hemolytic in nature.

For the patient with anemia, a well-balanced diet should be given. The classical experiments of Whipple and his coworkers<sup>137</sup> established the basis for present knowledge of the effect of various foods on hemoglobin production. These ultimately led to the discovery of the value of liver therapy for pernicious anemia and may be regarded as having initiated the modern era of hematology by stimulating the shift in emphasis from physiologic to physiologic investigation. In dogs made anemic by bleeding and fed almost-bread ration deficient in a number of nutritional factors, animal organs such as liver, kidney, and chicken gizzard were found to be the most efficient of all the foods in promoting hemoglobin regeneration. Dairy products were the least effective, whereas vegetables were second only to liver. Fruits, such as apricots, peaches, prunes, raisins, fresh grapes, and apples, were found to be of greater value than the chlorophyll-containing vegetables such as spinach and beet greens. The value in these foods can now be ascribed to their content of protein, as well as folic acid, vitamin B<sub>12</sub> and other vitamins, and iron and other minerals.

Liver is a complex mixture rich in proteins and their subunits, as well as iron, and vitamins including folic acid and cobalamin. It is the mainstay of treatment for pernicious

anemia, first by the oral route and later by the parenteral administration of concentrates, liver therapy is now outmoded. For the treatment of pernicious anemia, it has been replaced by injections of crystalline cobalamin (Chapter 14, page 590). Except for exceedingly rare cases of a peculiar type of anemia,<sup>68</sup> liver may now be considered only as a part of a balanced diet, rather than a specific therapy for anemia.

Vitamins are obtained in sufficient amount from the balanced diet; except for the presence of specific nutritional deficiency, the addition of supplementary amounts will not accelerate the recovery from anemia. Thus, the administration of ascorbic acid is associated with improvement when scurvy is present, but it does not appear to serve a specific function in hematopoiesis. Even though pyridoxine deficiency may be induced experimentally in animals, and "pyridoxine-responsive" anemia is known in humans (page 680), the latter is not due to deficiency of pyridoxine. Amounts of vitamin B<sub>6</sub> adequate for human nutrition are obtained from a diet that is otherwise satisfactory with reference to protein, vitamins, and minerals. Accordingly, the administration of pyridoxine and other vitamins and minerals as a nonspecific nutritional supplement is unnecessary, and involves a needless expense.

Foods such as pastry and "soft drinks," which are high in carbohydrates but deficient in the substances mentioned above, should be avoided as they tend to displace other, balanced foods. Indeed, just as nutritional anemia may be induced by faulty diet or cooking,<sup>30,64,72</sup> so may the recovery from anemia be hampered. The consistency of the food and the number of feedings must be adjusted to the state of health of the patient. Tasteful preparation and service of the food will avoid monotony of diet and help the ill patient with anorexia overcome the inclination to accept no more than a minimal diet.

### Other Measures

Other measures in the general treatment of anemic patients are similar to the measures

employed in the treatment of any patient with an acute or chronic disease. Physical rest, fresh air, sufficient sleep, and freedom from anguish are valuable and important. Physical exertion to the point of actual fatigue should be avoided, but graded exercise should be prescribed according to the patient's strength and general well-being. It must be remembered that when anemia is marked the cardiac reserve may be minimal. Particularly in elderly patients, angina pectoris, congestive heart failure, or cardiac arrhythmias may be present and would require treatment. Consideration should be given to the physical strain placed upon the patient by diagnostic studies, such as sigmoidoscopy, barium enema, gastroscopy, or bronchoscopy. In some instances, in order to avoid cardiac complications it is necessary to transfuse the patient with concentrated red cells before undertaking such studies. In general, with the successful treatment of anemia, respiratory and circulatory symptoms will be alleviated and angina pectoris may vanish as the hemoglobin concentration rises. Digitalis glycosides and diuretic agents, which may be necessary for the control of congestive heart failure when anemia is present, may not be required after the anemia has been relieved.

### Administration of Substances Specifically Lacking

#### *General Considerations*

In the discussion of the classification of anemia, it was pointed out that, of the macrocytic anemias, the type characterized by megaloblasts in the bone marrow most frequently results from a lack of vitamin B<sub>12</sub> or folic acid. The clinical syndromes associated with these deficiencies are listed in the tables of this chapter and are discussed fully in Chapters 14 and 15. The development of our knowledge concerning the role of these substances in hematopoiesis has been described in Chapter 4.

The detection of hypochromic-microcytic anemia presents a special challenge to the physician. The clinical syndromes of iron-

deficiency anemia are discussed in Chapter 17. In most cases, chronic blood loss is implied and the source must be discovered. Simply administering iron is not enough. The finding of such an anemia is often the first signal of pathologic blood loss, as is fully explained there (page 641).

When anemia is due to deficiency of a substance essential for erythropoiesis, such as vitamin B<sub>12</sub>, folic acid, or iron, administration of the agent lacking will be followed by a very gratifying therapeutic response. Of paramount importance, however, is the understanding that these substances exert a salutary, therapeutic effect in the treatment of anemia only when deficiency of the specific substance is the limiting factor in erythropoiesis. Furthermore, only rarely will deficiency of two or more of these substances occur simultaneously. Successful treatment requires foreknowledge of the correct diagnosis, and choice of the correct agent.

### *Therapeutic Response*

Descriptions of the therapeutic response to the administration of substances that are specifically lacking, such as vitamin B<sub>12</sub>, folic acid, and iron, are given in the chapters dealing with these deficiencies, namely, Chapters 14 and 17. There also the importance of replenishing body stores of these essential nutrients is discussed and therapeutic regimens are outlined. Here it need be stated only that, in the vitamin-deficiency states, the response to administration of the substance that is lacking is extraordinarily rapid and occurs following administration of very small quantities of that substance. The response to iron therapy is less dramatic but equally specific.

**THERAPEUTIC TRIAL.** An optimal hematologic response to the administration of a vitamin or mineral constitutes the ultimate demonstration that deficiency of the administered nutrient was the limiting factor in erythropoiesis. This principle underlies the use of the therapeutic trial for diagnostic purposes. Although specific assays for serum vitamin B<sub>12</sub>, folate, and iron (Chapters 14 and 16)

have largely replaced the diagnostic therapeutic trial in clinical practice, there still are circumstances in which trials may be useful. For one thing, they are readily available to physicians who may not have access to laboratories performing the more complex assays. For another, serial trials may help to sort out the relative importance of multiple deficiencies in complex cases.

In conducting a therapeutic trial, it is important to establish baseline conditions by performing determinations of reticulocyte counts daily and measuring VPRC or hemoglobin two or more times, preferably for 7 to 10 days before the agent to be tested is given. Then the agent is administered, usually in a minimally effective dose, and daily measurements of reticulocytes and repeated determinations of VPRC are continued. The trial is considered to be positive if a significant reticulocytosis occurs within 10 days after administration of the agent was commenced. Values for the expected peak reticulocyte count at various degrees of anemia are given in Table 14-7 (page 594) for the megaloblastic anemias; the expected rate of VPRC increase appears in Figure 14-11 (page 595). In the case of iron therapy in iron-deficiency anemia, the increase in reticulocytes is generally slower to appear and not as great as in the megaloblastic anemias (Chapter 17).

The use of minimally effective doses is especially important in therapeutic trials in the megaloblastic anemias because patients with vitamin B<sub>12</sub> deficiency will respond to large doses of folate, and those with folate deficiency may respond to large doses of vitamin B<sub>12</sub>.<sup>65,147</sup> The minimal effective dose for vitamin B<sub>12</sub> is 1 to 2 µg per day<sup>125</sup> and that for folate is 200 µg per day. These doses induce a hematologic response only in patients deficient in the administered vitamin.

**DOUBLE RETICULOCYTE RESPONSE TECHNIQUE.** This provides a very useful method for clinical investigation.<sup>89</sup> It may be used to establish the minimum dose of a substance that can achieve an optimum hematologic response, or to evaluate the presence of concomitant deficiencies of two substances. After

the reticulocytes have returned to normal following the first therapeutic trial, a second trial is conducted with either a larger dose of the same substance or a particular dose of another substance. If a second reticulocyte response occurs, and the second trial entailed a higher dose of the same substance, it implies that a suboptimal dose was used in the first trial. If the second trial employed a different compound, either a deficiency of both compounds is implied or the response to the first substance was nonspecific.

### "Shotgun" Treatment

"Shotgun" treatment consists in the indiscriminate use of several therapeutic agents simultaneously, each of which is effective individually in specific types of anemia, without first determining their applicability to the case at hand. Also in the treatment of anemia, it refers to the use of pharmaceutical mixtures which include vitamins and minerals, deficiency of which diligent study has failed to establish as a cause of disease in humans.<sup>142</sup> Iron, folic acid, vitamin B<sub>12</sub>, riboflavin, pyridoxine, niacin, copper, possibly ascorbic acid, adrenal corticosteroids, androgenic steroids, and antibiotics have specific indications for treatment of anemia. However, the general use of fixed combinations of multiple agents for therapy is irrational. Combinations of agents deprive the physician and the patient of the important evidence that a response to specific therapy can provide, namely, the confirmation of the diagnosis. A combination of several therapeutic agents is sometimes prescribed out of ignorance of the diagnosis in the hope that one of the ingredients will hit the mark (ie, bring about an increase in the hemoglobin concentration) even though one did not know where to aim; hence, the term "shotgun therapy." Sometimes they are prescribed for their principal ingredient, usually iron, without an appreciation on the physician's part that they are, in fact, a shotgun mixture.

The use of multiagent, "shotgun therapy" is strongly condemned. Merely bringing about an increase in the hemoglobin concen-

tration is an unsatisfactory result and constitutes inadequate therapy. A plan for further management is an essential ingredient of the treatment of anemia. That plan depends upon knowledge of the underlying cause of the anemia.

In addition to the medical considerations against the use of "shotgun" preparations, the physician must also be concerned with their excessive cost which is in part a reflection of the inclusion of ingredients that are unnecessary for the treatment of the patient at hand.<sup>142</sup> Medicolegal considerations also cannot be ignored.

### Splenectomy

Splenectomy is of greatest value in the treatment of the patient with anemia of hereditary spherocytosis (page 756). It is sometimes helpful, but not curative, in the hemolytic anemia of pyruvate kinase deficiency (page 773), and in some patients with acquired hemolytic anemia who have a positive reaction to Coombs' antiglobulin test, when adrenocorticosteroid hormones are required in such large doses and for so long a time that their use is impractical. Splenectomy is sometimes of benefit in aplastic anemia (page 1763), and rarely so in thalassemia (page 869). In Banti's syndrome, congestive splenomegaly with anemia, leukopenia or thrombocytopenia, splenectomy may be undertaken with the possibility of improvement, provided all other causes of the splenomegaly and cytopenias have truly been excluded by careful evaluation (Chapter 46). Too often, a diagnosis of "Banti's disease" or "hyper-splenism" is attached when in reality the anemia and splenomegaly are due to lymphoma or other disease in which splenectomy would not relieve the anemia. In Felty's syndrome, splenectomy does not appear to be of lasting benefit. In selected patients with myeloid metaplasia with splenomegaly, splenectomy is helpful; but in others, in whom the spleen plays a major role in compensatory, extramedullary hematopoiesis, splenectomy could, at least theoretically, aggravate the anemia (Chapter 57).

### Hormones

Of the hormones, the use of thyroxine or equivalent compounds will be curative in the anemia of myxedema, but not beneficial for anemia of other causes. Cortisone, prednisone, and related *adrenal glucocorticoid hormones* will relieve the anemia of Addisonian adrenal insufficiency, even when given in small amounts. However, their more frequent application is in the treatment of acquired hemolytic anemia of the auto-antibody type (page 914). Here, given in pharmacologic rather than physiologic doses, they are very potent in the control of anemia. The same hormones are valuable in the induction of a remission of acute lymphoblastic leukemia, thereby allowing recovery from the accompanying anemia. They have been considered to stimulate erythropoiesis because reticulocytosis may follow their administration (even in pernicious anemia in relapse), and erythroid hyperplasia of the bone marrow may develop, nucleated red cells may appear in the circulation,<sup>144</sup> and polycythemia may ensue. However, in disease states with anemia, other than those noted above and in certain cases of "pure red cell aplasia," a useful erythropoietic effect cannot be obtained with prednisone or related compounds.

Glucocorticoids are particularly useful when they are needed only for short periods of time. The side effects of long-term therapy are many (Table 13-6) and potential hematologic benefit must be weighed against undesirable manifestations.

*Estrogens* may relieve the anemia in postmenopausal patients with metastatic carcinoma of the breast, and in patients with metastatic carcinoma of the prostate. *Androgens* may likewise be used in metastatic carcinoma of the breast, when estrogens fail. Oxymetholone, testosterone enanthate, and other congeners of androgenic hormones have been used to stimulate erythropoiesis in the bone-marrow failure of aplastic anemia, congenital hypoplastic anemia of children, myelofibrosis, paroxysmal nocturnal hemoglobinuria, and other conditions.<sup>63,110,283</sup> Even though their major action is via eryth-

**Table 13-6. Side Effects of ACTH and Steroid Therapy***Features seen in Cushing's syndrome**Electrolytic*

Fluid retention—edema

Hypokalemia

*Distiguring*

Round facial contour, plethoric appearance

Humps—cervicodorsal ("buffalo")

anterocervical ("turkey")

Acne, pigmentation, hirsutism &amp; baldness

Kyphosis

*Disquieting*

Muscle wasting

Hypertension

Glycosuria

Menstrual disturbances impotence

*Other side effects**Enjoyable*

Appetite increase

Mild euphoria

*Disturbing*

Insomnia

Polyuria

Headache

Thin skin, striae, easy bruising poor healing

Myopathy

*Alarming*

Epigastric discomfort (peptic ulcer)

Intercurrent infections

Osteoporosis

Psychiatric symptoms

Convulsions

ropoietin, and erythropoietin excretion is already high in such patients, useful therapeutic successes have been claimed. The underlying disorders are not improved except, perhaps, in aplastic anemia.

### Chemotherapy and Irradiation

*Chemotherapy* can relieve the anemia in leukemia, Hodgkin's disease, and related conditions when such treatment influences the disorder causing the anemia. Thus, the administration of busulfan in chronic myelocytic leukemia, adrenal glucocorticoid hormones and metabolic antagonists in acute leukemias, and nitrogen mustard, or other alkylating agents together with other compounds, in Hodgkin's disease (page 1559) may all bring about disappearance of anemia as well as relief of other manifestations of the disease in question. In other conditions, such as the anemia associated with metastatic malignant tumors of the bone marrow, chemotherapy is less often effective but may have a dramatic effect. Details of the treatment of these and related disorders are discussed separately in the specific chapters dealing with these diseases.

*Irradiation* therapy from external sources such as  $^{60}\text{Co}$ , roentgen therapy, the linear accelerator, or internal irradiation with radioactive phosphorus may be used to advantage in selected anemic patients with lymphoma,

leukemia, or sometimes metastatic carcinoma. In these subjects, judicious treatment can bring about temporary or even long-term remissions, and thereby control the anemia. On the other hand, too vigorous treatment can add problems of drug- or radiation-induced bone marrow failure.

## Anemia in Pregnancy

Anemia in pregnancy is a multifaceted subject. It must first be appreciated that, beginning in the sixth week of pregnancy, the plasma volume increases disproportionately to the red cell mass. The plasma volume reaches a peak value at 24 weeks,<sup>201</sup> or later.<sup>202</sup> On the average, the maximal value is 43% greater in pregnant women than in nonpregnant women<sup>198</sup> (Table 13-7). Thus, even if the red cell mass did not change, a "dilutional anemia" would result. Indeed, a reduction in the hematocrit and hemoglobin concentration is evident by the sixth or eighth week of normal pregnancy<sup>165,203,214</sup> and progresses until the sixteenth or twenty-second week when a new equilibrium is established.<sup>198,202</sup> These values usually stabilize, with the hematocrit at 0.32 to 0.34 l/l and the hemoglobin at 11 g/dl. Red cells remain normochromic and normocytic unless deficiency of iron or folate supervenes. When the hemoglobin concentration is less than 10.4 g/dl, a true reduction in red cell mass

Table 13-7. Plasma Volume and Red Cell Mass in Pregnant and Nonpregnant Women\*

	<i>n</i>	Nonpregnant		<i>n</i>	Pregnant		Increase during	
		mean	SD		mean	SD	Pregnancy	
		(ml)	(ml)		(ml)	(ml)	(ml)	(%)
RBC mass	102	1367	± 137	294	1711	± 100	344	25
Plasma volume	440	2566	± 262	955	3680	± 391	1114	43

\* Collected data from reference 173. Only direct measurements of plasma volume or red cell mass are included. Inferred values of one computed from measurement of the other, are not included.

*n* refers to the number of subjects studied. SD to standard deviation.

is likely to be present, but, because of variations in the hydremia, a fixed dividing line between the normal and abnormal is more difficult to place in pregnancy than under other circumstances.<sup>178</sup> The relationship between the hematocrit of venous blood and the hematocrit of the body as a whole is probably unchanged in pregnancy,<sup>213,217</sup> although some reports indicate that it is variable.<sup>32,211,228</sup>

Masked by the dilutional effect, there is an actual increase of about 17 to 25% in the red cell mass during pregnancy.<sup>173,193,214</sup> (Table 13-7). A greater increase in red cell mass occurs in normal pregnancy if iron supplementation is taken.<sup>178</sup> Ferrokinetic studies have shown accelerated erythropoiesis during pregnancy, supporting the concept of an actual increase in red cell mass.<sup>215,218</sup>

Data regarding a reduction in plasma volume and an increase in hemoglobin concentration during the final six weeks of normal pregnancy are conflicting.<sup>173,178</sup> Measurements of plasma volume made in late pregnancy with the patient supine, as contrasted to those made with the patient in the decubitus position, are subject to error attributed to interference with the circulation imposed by the gravid uterus.<sup>173</sup> With this in mind, conflicting reports regarding changes in plasma volume near term may only represent variations within the error of measurement, or random occurrences.<sup>208,209,214</sup>

The mechanisms underlying these changes in blood volume and red cell mass are obscure. An increase in urinary and plasma

erythropoietin in the last two trimesters has been documented.<sup>201</sup> Plasma erythropoietin has been reported to be 30 to 35% greater in pregnant women near term, than in nonpregnant women, and it decreases promptly after delivery.<sup>205</sup> Perhaps of significance are the observations made in the pregnant mouse, whose changes in blood volume and red cell mass parallel those of the human.<sup>192</sup> Erythropoietic activity in serum is greater in the pregnant than in the nonpregnant mouse.<sup>191</sup> Human placenta lactogen stimulates erythropoiesis in the mouse,<sup>192,193</sup> but it is not known whether that hormone is responsible for the increased red cell mass in the pregnant woman.

In the puerperium, the blood volume returns to normal within one to three weeks,<sup>181,202,228</sup> partly reflecting blood loss at delivery<sup>218</sup> and partly the return of the plasma volume to normal.<sup>178</sup>

Iron deficiency not infrequently complicates the picture<sup>178</sup> (Chapter 17). Limited iron reserves even before pregnancy are commonplace<sup>210,216,223</sup> and the additional iron requirements of pregnancy impose a negative iron balance<sup>167,210,229</sup> unless supplemental iron is provided.<sup>178</sup> Frank iron deficiency is a common cause for anemia in pregnancy. The usual criteria for diagnosis apply (Chapters 16 and 17), including, in particular, reduction in the serum iron concentration to less than 60 µg/100 ml (10.7 µmol/l), occurring together with a transferrin saturation of less than 16% and, in the more severe cases, hypochromia of the red cells and a

reduction in the MCHC. However, even in apparently healthy women, during pregnancy there is a progressive decline in the serum iron, and an increase in the serum total iron-binding capacity and in the erythrocytic free protoporphyrin.<sup>178,180</sup> Findings that are associated with iron deficiency.<sup>142,99</sup> Dietary supplementation with 78 mg of elemental ferrous iron daily during pregnancy increased the hematocrit, hemoglobin concentration, and red cell mass during pregnancy, these values rising to nearly twice those found in similar, apparently healthy women who did not receive the supplement.<sup>178</sup> At term, the mean hemoglobin concentration was 12.4 g/dl in those who received the iron supplement, and 10.9 g/dl in those not receiving it.<sup>178</sup> In Bantu women whose diet is habitually high in iron, a significant change in serum iron does not occur and iron-deficiency anemia does not develop during pregnancy.<sup>185</sup>

*Macrocytic anemia of pregnancy* is often megaloblastic and in most cases is due to deficiency of folic acid.<sup>168,183,186,196,199,200,225</sup> When it occurs, megaloblastic anemia is found most often in the third trimester, or shortly after delivery.<sup>183,221</sup> Folate requirements are higher during pregnancy, and the diets of many pregnant patients are insufficient to meet the increased need.<sup>172,177</sup> Although folate deficiency occurs most often in economically deprived patients, this consequence of poor eating habits is not confined to the poor.<sup>174,177,183,186,221</sup> In pregnant adolescents in particular, the diet may prove to provide an inadequate source of folate, regardless of the economic class.<sup>177</sup> In uncomplicated pregnancies, the gastrointestinal absorption of food folate (polyglutamate) and of folic acid (monoglutamate) is normal.<sup>207</sup> Folate deficiency in pregnancy is relatively common, although its frequency depends upon the population studied. Reports indicate that it varies from 2 to 3% up to about 50% of pregnant patients.<sup>174,190,200,221</sup> Not all patients in whom the serum concentration of folate is low will develop megaloblastic anemia. Of those who do, frequently the serum folate concentration was low earlier in pregnancy.<sup>221</sup> Dietary supplementation with

about 0.3 mg of folic acid daily during pregnancy will reduce the occurrence of megaloblastic anemia to about 0.7% of all pregnant women.<sup>190,221,231</sup> whereas a supplement of only 0.1 to 0.2 mg daily is not adequate to maintain serum folic acid concentration at normal levels.<sup>168,230</sup> A supplement of 0.45 mg daily results in supranormal values for serum folate,<sup>230,231</sup> but, even with this high a supplement, megaloblastic anemia may occur in an occasional pregnant patient in whom the course is complicated by urinary tract infection or by hemorrhage.<sup>231</sup> Nevertheless, since true pernicious anemia due to lack of intrinsic factor and vitamin B<sub>12</sub> does occur in pregnant patients,<sup>204</sup> the considerations mentioned elsewhere (page 553), regarding masking of pernicious anemia with folate doses of 0.40 mg/day or more, apply. Accordingly, daily supplements of 0.3 mg of folic acid, but not more, are generally prescribed in pregnancy.

A positive association between bacteriuria and folate deficiency has been found in pregnant patients.<sup>204</sup> In experimental systems, infection may induce folate-deficient megaloblastic anemia.<sup>208</sup> Other complications of pregnancy such as increased incidence of abruptio placentae, uterine bleeding, prematurity, reduced birth weight, and fetal malformations<sup>175,188,189,195,204,225</sup> have been attributed to folate deficiency, but some large clinical studies do not confirm such a relationship.<sup>177,219,224,231</sup>

Malabsorption (Chapter 14) may cause, or be associated with, megaloblastic anemia of pregnancy, even in nontropical areas.<sup>196</sup>

Rarely, the folate-deficient pregnant patient may present with *erythroblastopenia* and a bone marrow appearance so closely simulating acute leukemia, because of the predominance of granulocyte precursors, that even hematologists may diagnose it as such. In one case, the findings suggesting leukemia were so convincing that 6-mercaptopurine was prescribed for four months.<sup>197</sup> If one is aware that confusion between these diagnoses has occurred before, and applies the strict criteria given elsewhere in this book (Chapter 41), difficulty should be avoided. Extra effort



is always appropriate to exclude the possibility of a treatable, although seemingly unlikely diagnosis, when the alternative is an incurable disease.

Pregnant patients are, of course, also liable to the same diseases that afflict other women. Some chronic anemias present special hazards to the pregnant patient. Sick cell disease<sup>181</sup> and other conditions in this category are considered elsewhere in this book.

## Features of Anemia Unique to Infants and Children

The first days and months of life require special hematologic consideration for several reasons. (1) certain diseases are unique to the newborn; (2) normal values differ from those of older children and adults, and significant changes occur in the first week after delivery; (3) the enzyme content and activity of the circulating red cells, and their content of fetal hemoglobin, are not identical with those found later in life; (4) the responsiveness of the hematopoietic system of the newborn and of infants and children is not the same as in adults; (5) nutritional factors may have a great effect upon the hematopoietic system; (6) growth imposes increased requirements for iron and other factors required for erythropoiesis; and (7) many congenital and hereditary disorders first make their appearance early in life.

Several special features characterize the erythrocytes of the newborn. Erythropoiesis is accelerated as compared with the adult. This is reflected in reticulocytosis, macrocytosis, and the presence of nucleated red cells in the circulation. Erythrocytes of the newborn remain in the circulation for an average of 85 to 91 days, as compared with 120 to 127 days in the adult.<sup>263,289</sup>

### Red Cell Enzymes

In the relatively immature erythrocytes of the newborn, the activity of certain enzymes is increased.<sup>293</sup> These enzymes include hexo-  
glucose-6-phosphate dehydrogenase (G6PD), 6-phosphogluconic acid dehydro-

genase, aldolase, phosphoglyceric acid kinase, pyruvate kinase, and lactate dehydrogenase (LDH).<sup>276,286</sup> The activity of G6PD is greater at birth than in later life, but decreases during the third to ninth weeks of life. It rises again in the subsequent eight weeks.<sup>268</sup> On the other hand, the activity of certain other enzymes is decreased at first, and only later attains the rates found in adults. Thus, phosphofructokinase<sup>276</sup> and acetylcholinesterase activity (AChE) are lower at birth than in the red cells of adults. The latter decreases even further and then increases in subsequent weeks, to reach, by the fourth month of life, values that are found in adults.<sup>268</sup> The low activity of NADP-dependent *methemoglobin reductase* (diaphorase) plays a role in the infant's increased susceptibility to the development of methemoglobinemia (page 1011). Carbonic anhydrase, catalase, and glyoxalase are also low in the erythrocytes of the newborn.

*Instability of ATP* is a feature that is even more marked in the red cells of the premature infant than in infants born at term.<sup>277</sup> The mechanical fragility of red corpuscles of the newborn is increased. There also are differences in the antigenic components, particularly of the Ii blood group system (page 458).

After the first few days of life, the rate of synthesis of hemoglobin slows down greatly and does not accelerate again until the infant reaches the age of 1½ to 2½ months.<sup>263,268</sup> (page 56).

### Hematopoietic Equilibrium

The hematopoietic equilibrium of the newborn and infant is less stable than that at later ages. As a result, the effect of any given stimulus, such as infection, may differ not only quantitatively, but also qualitatively. Leukocytosis may be striking, and myelocytes or even myeloblasts may be found in the circulation instead of a slight or moderate "shift to the left" which may occur in adults in similar circumstances. Anemia may be more profound, and nucleated red cells may appear in the circulation, whereas only polychromatophilia and reticulocytosis would de-

velop in an adult. Lymphocytosis develops frequently and to a more striking degree, and the spleen, lymph nodes, and liver become enlarged more readily than in adults.

These differences in the response of the hematopoietic system explain some of the observations which led to the term "anemia pseudoleukemica infantum," introduced by von Jaksch<sup>290</sup> to describe certain anemic conditions in infants. No doubt, this represented the consequence of a variety of factors including nutritional deficiencies, gastrointestinal disturbances, syphilis, tuberculosis and a number of other infections, iron deficiency, and even thalassemia. The term was applied to patients under three years of age with splenomegaly, hepatomegaly, lymphadenopathy, and anemia with marked anisocytosis, poikilocytosis, erythroblasts and macro-normoblasts in the circulation, extreme leukocytosis, and relative lymphocytosis. This is mentioned to emphasize that, when compared with the blood of adults, the morphologic features of the blood in infants may be more complex or bizarre in response to relatively commonplace disorders.

### Hereditary and Prenatal Factors

*Genetic constitution* as regards the hematopoietic system of the fetus may, both directly and indirectly, govern the fetus' survival. Certain genetic determinants, by direct effect, may be lethal. Thus, homozygosity for *alpha thalassemia* markedly restricts the capacity to synthesize alpha chains of hemoglobin. When completely expressed, this precludes synthesis of even fetal hemoglobin (Chapter 26) and results in *fetal hydrops*. Homozygosity for the methemoglobins (Hb M) presumably would be a lethal anomaly (Chapter 31), for it would preclude reversible binding of oxygen by hemoglobin. Only the heterozygous forms have been identified in living persons. The same also may be true for hereditary telangiectasia (page 1144).

Inheritance acts by an indirect mechanism in the *isoimmune diseases*. For example, in *erythroblastosis fetalis* resulting from Rh antigen incompatibility between mother and

fetus, the adverse consequences result from the antibodies produced by the mother that escape into the fetal circulation, there acting against the erythrocytes<sup>291</sup> (Chapter 27). The fate of the fetus depends upon the quantity and timing of the antibody production. Other, less serious forms of isoimmune hemolytic anemia operate similarly.

Factors concerned with the fetal environment and its unique relationship to the maternal circulation and, sometimes, to a twin pregnancy, may also act to produce anemia in ways which do not occur in adults. Such causes for anemia in the newborn are listed in Tables 13-8 and 13-9. These are less likely to be lethal than the aforementioned genetic and isoimmune disorders, provided they are recognized at once and treated. As in adults, the causes may be conveniently grouped into those due to hemorrhage, hemolysis, and erythroid hypoplasia or underproduction.

Constitutional aplastic anemias are discussed in Chapter 56 (page 1770).

**Table 13-8. Causes of Anemia in the Newborn**

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I Hemorrhage (refer to Table 13-9)
II Hemolysis
A 'Isoimmune disease'
B Infections*
1 Pyogenic, sepsis
2 Cytomegalic inclusion of disease
3 Congenital rubella
4 Congenital syphilis
C Hereditary spherocytosis†
D Hereditary elliptocytosis†
E Pyruvate kinase deficiency, and other disorders of glycolysis†
F Hereditary non-spherocytic hemolytic anemia not otherwise classified
G Glucose-6-phosphate dehydrogenase deficiency†—drug-induced anemia
H Autoimmune
I Galactosemia
J Infantile pyknocytosis
K $\alpha$ -Thalassemia†
III Erythroid hypoplasia (Blackfan-Diamond syndrome)†

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\*Acquired

†Hereditary

‡Usually not symptomatic at birth

**Table 13-9. Causes of Hemorrhagic Anemia in the Newborn**

- 1 Feto-maternal hemorrhage\*†
- 2 Feto-fetal transfusion\*  
( "twin-transfusion syndrome" )
- 3 Hemorrhage from umbilical cord or placenta‡
  - a Rupture or tear
  - b Placenta previa
  - c Abruptio placentae
- 4 Trauma‡
  - a Cephalohematoma
  - b Rupture of liver, spleen
  - c Retroperitoneal bleeding
- 5 Gastrointestinal‡
- 6 Disorders of coagulation

\*Antepartum

†Intrapartum

‡Postpartum

### Hemorrhage

The general considerations regarding anemia due to acute and chronic blood loss mentioned earlier in this chapter apply to the newborn. Special attention must be given to the small blood volume of the infant. In a newborn, the blood volume averages 80.4 ml/kg.<sup>275</sup> The loss of 30 to 50 ml of whole blood will produce pallor, anemia, and severe distress. Loss of a greater quantity of blood may produce shock. Similarly, small losses (by absolute measure) of fluid may result in significant dehydration and alter the hematocrit accordingly; parenteral administration of blood or fluids will likewise have a relatively greater effect than in adults. The causes of hemorrhagic anemia in the newborn are given in Table 13-9.

*Feto-maternal hemorrhage*<sup>254,280</sup> of more than 40 ml is sufficient to produce an anemic infant. This occurs in nearly 1% of pregnancies.<sup>255</sup> To a lesser degree, *feto-maternal transfusion* occurs in about half of all pregnancies.<sup>255-294,295</sup> However, in only 8 or 9% does the amount exceed 0.5 ml.<sup>235</sup> The presence of fetal erythrocytes in the maternal circulation can be demonstrated by the technique of Kleihauer et al.<sup>270</sup> On a blood film prepared from the mother, hemoglobin A is eluted from the red cells at an acid pH, whereas fetal hemoglobin remains. The latter

may be seen on microscopic examination of the stained blood film.<sup>285</sup> The possibility of other conditions resulting in an increase in hemoglobin F in maternal red cells must be excluded for this test to be applicable. Differential agglutination is a less sensitive approach to the detection of fetal erythrocytes in the maternal circulation,<sup>254,265</sup> and chemical estimation of the hemoglobin F content in maternal blood will only suffice to detect the largest feto-maternal hemorrhages. Repeated fetal blood loss by this mechanism over some period of time<sup>291</sup> can result in iron-deficiency anemia in the newborn.<sup>280,282</sup>

The "*twin transfusion syndrome*,"—transfusion from one fetus to another via an arterio-venous communication or other shunt, in a monozygotic twin pregnancy—results in anemia in one and polycythemia in the other twin.<sup>257,274</sup> It is not rare, having been found in 19 of 130 monozygotic twin pregnancies.<sup>281</sup> The effects may be severe. If a still-birth does not result, listlessness or shock is present at birth in the anemic member,<sup>291</sup> and hypertension, cardiomegaly, hepatosplenomegaly, polyhydramnios, and later congestive heart failure occur in the plethoric member. Death may occur in the neonatal period unless treatment is given promptly.<sup>281</sup> The syndrome should be suspected if the hemoglobin concentration in twins of the same sex differs by more than 3.3 g/dl at birth.<sup>281</sup> If such difference is found, the placenta should be examined for an arterio-venous communication, or a laceration.

Hemorrhage from rupture or tear of the *placenta or umbilical vessels* has been secondary only to hemolytic disease as a cause of anemia in the newborn.<sup>269</sup> Now, with the decline in frequency of Rh-induced hemolytic disease (Chapter 27) in the newborn, hemorrhage from rupture or tear will become even more important. The infant may be listless, pale, or in shock. The diagnosis is made on inspection of the umbilical cord and placenta.

### Hemolytic Disease

In the newborn, hemolytic disease is most often of the isoimmune type (Chapter 27)

although most of the inherited and some of the acquired forms of hemolytic disease also occur (Table 13-8).

In contrast to the characteristic findings in the adult with hemolytic disease, the infant is usually free of jaundice at birth, despite accelerated red cell destruction. In the next few days of life, jaundice appears and, compared with the findings in adults, it increases out of proportion to the rate of destruction of red cells.

There are unique features of the bilirubin catabolism in the fetus and newborn which affect the clinical presentation and consequences of hemolytic disease in the fetus and in the newborn. The liver is deficient in *glucuronyl transferase* activity until the infant is a few days old.<sup>272</sup> As a result, the normal conversion of bilirubin to the diglucuronide (Chapter 5) is impaired and excretion of the catabolic products of hemoglobin via the normal biliary pathway is reduced. During intrauterine life, the deficiency of glucuronyl transferase does not present a serious problem, since bilirubin readily traverses the placenta.<sup>286</sup> It is then metabolized and excreted by the mother so effectively that the newborn is free of jaundice even when there is hemolytic disease with accelerated destruction of red cells. The situation changes abruptly at birth with the loss of the maternal pathway. Bilirubin accumulates in the plasma, bound to albumin. For every gram of hemoglobin catabolized, about 35 mg of bilirubin result. This quantity is distributed in the smaller extracellular fluid space of the infant, resulting in a greater increment in bilirubin concentration than would occur in the adult. Until bilirubin glucuronide excretion is established, the bilirubin concentration rises in the serum. Degradation of the diglucuronide by intestinal  $\beta$ -glucuronidase activity in the intestine allows some bilirubin to be reabsorbed. The combination of these factors causes the serum bilirubin concentration to rise and clinical jaundice to worsen more quickly than would be expected under parallel circumstances of hemolytic disease in the adult. If the serum concentration of bilirubin exceeds 20 mg/dl, there is a danger of

kernicterus<sup>264</sup> and its disastrous consequences.<sup>265</sup>

*Genetically determined hemolytic diseases* may be symptomatic in the newborn (Table 13-8), although sickle cell disease, thalassemia, and hereditary spherocytosis usually are not. However, when a hemolytic anemia with spherocytic red cells is present at birth, hereditary spherocytosis must be considered as well as isoimmune disease (ABO incompatibility) (Chapters 21 and 27). It must be remembered that in about one sixth of the patients with hereditary spherocytosis, the disorder cannot be demonstrated in the parents even though it is believed to be transmitted as an autosomal dominant. Hence, the differentiation of genetically determined disorders may not always be simple.

*Drug-induced hemolytic disease* with hyperbilirubinemia has been reported in infants, particularly premature, who had been given 5 or more milligrams of a water-soluble vitamin K preparation,<sup>250</sup> intended as prophylaxis against hemorrhagic disease of the newborn. The adverse effect can be avoided if the dose is not greater than 1 mg.<sup>251</sup> *Glucose-6-phosphate dehydrogenase deficiency* may be associated with hyperbilirubinemia in some infants<sup>290</sup> or with drug-induced hemolytic disease. The latter may even result from drug exposure of the infant when in utero.<sup>292</sup> These disorders are fully discussed elsewhere in this volume.

## Nutritional Considerations in Anemia in Infants and Children

The nutritional requirements of the infant and child are especially important in the light of the rapid growth which must occur. Iron reserves are influenced by the timing of the tying of the umbilical cord. If the cord is tied too early, the infant will be deprived of additional red cells from the placenta, which would provide not only additional blood volume but also additional iron reserve. The hemoglobin concentration at birth is higher than will be found later. Part of this excess

will contribute to iron reserve. After birth, iron requirements are provided by the diet which, if confined to milk, will be insufficient. These topics are discussed in detail in Chapter 17 (page 645), where the problems of the premature infant are also considered. Deficiencies of folic acid and protein also play important roles in anemia in childhood, as discussed in Chapter 14 (page 578).

It should be clear from the above that the differentiation of the causes of anemia in the newborn requires especially careful consideration of the family history, maternal health, and obstetric history. Infants, of course, cannot complain of weakness nor can they relate symptoms to direct attention to a specific organ system. The mother's observations are often helpful, but may be inadequate or misleading. Therefore, more than ordinary reliance must be placed upon careful observation and physical examination. With the exception of the factors already discussed, including the differences in normal values for the various age groups, the results of laboratory tests and their interpretation are as they would be in the case of adults.

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### *Macrocytosis and Macrocytic Anemias*

Significance of Macrocytosis  
Defective DNA Synthesis  
Accelerated Erythropoiesis  
Increased Membrane Surface Area  
Non-megaloblastic, Macrocytic Anemia  
Megaloblastic Anemia  
Laboratory Manifestations  
Disorders Associated with Megaloblastic Anemia  
Laboratory Tests Useful in Differential Diagnosis  
Diagnostic Approach to the Patient  
Management

THE macrocytic anemias are characterized by an increase in the average size of the erythrocyte. Usually the amount of hemoglobin in each cell is increased in proportion to size. Thus, in typical macrocytic anemia, the mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) are increased, and the mean corpuscular hemoglobin concentration (MCHC) is normal (Fig. 14-1).

Macrocytosis may also be detected by examination of the blood smear, since the average red cell diameter is increased; however, considerable patience is required to achieve precision in the microscopic detection of macrocytosis. Often, the change is accompanied by anisocytosis, which complicates the problem of estimating the average size. Furthermore, a change in diameter may not be apparent until the observer has examined many normal smears with the same microscope. Even experienced hematologists

should acquire the habit of comparing the abnormal smear with reference smears of normal blood. Otherwise, minimal to moderate deviations from normal may be overlooked.

In macrocytic anemia, most macrocytes are thicker than normal. Unless there is complicating iron deficiency the cells are well filled with hemoglobin. With the microscope, the increased thickness is perceived as a loss of central pallor. Because of these changes, the term "hyperchromic" was applied to the macrocytic anemias. This misleading term should be avoided because it suggests that the cellular concentration of hemoglobin is increased. In macrocytic anemia, as previously noted, hemoglobin content (MCH) is increased only in proportion to size; concentration, as measured by the MCHC, remains within normal limits.

### **Significance of Macrocytosis (Table 14-1)**

#### **Defective Deoxyribonucleic Acid (DNA) Synthesis**

The megaloblastic anemias are a group of disorders in which DNA synthesis is defective.<sup>9,15</sup> It has been proposed that this biochemical abnormality leads to a state of unbalanced cell growth, in which ribonucleic acid (RNA) and protein syntheses continue while DNA synthesis is retarded.<sup>1,15</sup> Thus, cytoplasmic components, especially hemoglo-

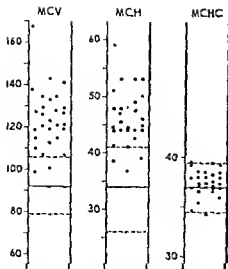


Fig 14-1. Erythrocyte indices in 28 patients with untreated or relapsed pernicious anemia. The dashed lines enclose the 95% confidence limits in normal subjects. MCV, mean corpuscular volume, MCH, mean corpuscular hemoglobin, MCHC, mean corpuscular hemoglobin concentration, ● males, ○ females. (From Hallberg,<sup>25</sup> courtesy of the author and Scandinavian Journal of Clinical and Laboratory Investigation.)

bin, are synthesized in excessive amounts during the delay between cell division. An enlarged cell is the final end-product of such a process.

### Accelerated Erythropoiesis

Macrocytosis frequently follows intense, erythropoietin-mediated stimulation of red cell production, such as may be induced by blood loss or hemolysis. At least three mechanisms appear to account for the increased cell size. First, the reticulocyte count increases, and normal circulating reticulocytes are about 20% larger than mature red cells.<sup>8,16</sup> Second, there is premature release of bone marrow reticulocytes ("shift" reticulocytes), which are larger and contain more RNA than normal circulating reticulocytes.<sup>6</sup> Finally, with intense stimulation, an erythroblast cell division may be skipped, a phenomenon that results in a macroreticulocyte that is approximately twice normal size.<sup>3,11,13</sup> The importance of the last mechanism in man has been questioned.<sup>6</sup> As macroreticulocytes cir-

culate, they lose water and hemoglobin intravascularly, possibly by cell fragmentation, until near normal size has been reached.<sup>5</sup>

### Increased Membrane Surface Area

In patients with liver disease (Chapter 19), a significant increase in cell volume results from one of the two mechanisms described above.<sup>7</sup> In liver disease, however, "thin macrocytes," which are defined as cells with an increased surface area ("macroplania"<sup>14</sup>) but without a corresponding increase in volume, also may be observed.<sup>2</sup> On blood smear, "thin macrocytes" are characterized by an increased diameter and a visibly enlarged area of central pallor. The characteristic "target cell" of liver disease is a thin macrocyte. Since the volume of such cells is normal, their presence has no effect on the erythrocyte indices.

The increased surface area of thin macrocytes is the consequence of excessive membrane lipids, especially cholesterol<sup>4,14</sup> but also phospholipids.<sup>10</sup> These alterations are reversible upon incubation in normal plasma and, conversely, can be induced in normal cells by incubating them in plasma from jaundiced subjects. The responsible plasma components probably are bile salts, which both inhibit cholesterol esterification and increase the rate of erythrocyte cholesterol uptake<sup>4</sup> (see Chapter 3).

Thin macrocytes with increased membrane lipid may also be observed after splenectomy. Apparently, membrane lipids normally are lost during the maturation of reticulocytes in the spleen<sup>12</sup>; in the absence of this organ, the lipid loss is of lesser magnitude.

## Non-megaloblastic Macrocytic Anemia

It is important to be aware of non-megaloblastic macrocytic anemias (Table 14-1, categories 2, 3, and 4) in order to avoid confusing them with the megaloblastic anemias. The non-megaloblastic anemias are only occasionally macrocytic; more often, they are normocytic. For that reason, the

**Table 14-1. Causes of Macrocytic Anemia**

- 1 Defective deoxyribonucleic acid (DNA) synthesis (the megaloblastic anemias—Table 14-3)
- 2 Accelerated erythropoiesis
  - a Hemolytic anemia
  - b Response to hemorrhage
- 3 Increased membrane surface area (thin macrocytes)
  - a Hepatic disease
  - b Obstructive jaundice
  - c Post-splenectomy
- 4 Of obscure origin
  - a Myxedema
  - b Hypoplastic anemia
  - c Acquired sideroblastic anemias
  - d Myelophthisic anemia

detailed discussion of these anemias is to be found in other sections of this book.

## Megaloblastic Anemia

### Laboratory Manifestations

#### The Blood

In the megaloblastic anemias, the degree of anemia ranges from levels barely compatible with life to levels indistinguishable from normal (Fig. 14-2). When symptoms of anemia are the presenting complaints the blood hemoglobin concentration usually is

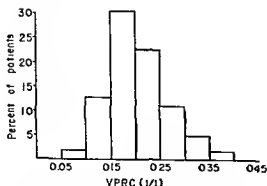


Fig 14-2. Severity of the anemia as measured by the volume of packed red cells (VPRC), in untreated patients with pernicious anemia (Prepared from data of Cox<sup>21</sup>)

less than 7 or 8 g/dl. However, if the patient is examined because of other symptoms, such as an underlying or unrelated disease, or if neurologic manifestations predominate, the degree of anemia is likely to be less pronounced.

When not complicated by iron deficiency, the anemia is found to be macrocytic and normochromic (MCHC within normal limits) (Fig. 14-1). The increase in MCV is to a large extent directly proportional to the degree of anemia, but other factors, such as the number of immature red corpuscles, also are important.<sup>15</sup> When the anemia is slight or moderate in degree, values of 95 to 110 fl ( $\mu^3$ ) are common (normal 82 to 92 fl ( $\mu^3$ )); when it is more severe the mean corpuscular volume may be 110 to even 160 fl and generally is found to range between 110 and 130 fl. The values for MCH will be found generally to range from 33 to 38 pg (normal 27 to 31 pg) when the anemia is moderate, and from 33 to even 56 pg when it is more severe.

The total leukocyte count may be within the normal range or below it. When the anemia is mild to moderate, the leukocyte count usually is  $3.0$  to  $6.0 \times 10^9/l$ . With more severe degrees of anemia, even greater reduction in the number of leukocytes may be observed ( $1.0$  to  $3.0 \times 10^9/l$ ).<sup>23</sup> The leukopenia usually is the result of absolute neutropenia.

The platelets generally are reduced in number, particularly when the VPRC is lower than 0.25 l/l. The platelet count may then be less than  $100 \times 10^9/l$ .<sup>56</sup> Bizarre forms, including giant platelets, may be found; the bleeding time may be prolonged; the blood clot may retract poorly; and even purpura, as well as retinal hemorrhages, may occur.<sup>60</sup>

### Morphologic Characteristics

The two principal findings on blood smear are hypersegmentation of the granulocytes and macroovalocytosis of the erythrocytes (Fig. 14-3). The leukocyte changes are particularly noteworthy because they are among the first hematologic abnormalities to appear



Fig 14-3 Photomicrograph of blood smears from patients with pernicious anemia in relapse showing (above) the macrocytosis, anisocytosis, and poikilocytosis, and (below) a multisegmented neutrophilic leukocyte (Wright's stain,  $\times 1040$ )

as the megaloblastic state develops.<sup>40</sup> Normally, the nuclei of circulating, segmented neutrophils have less than five lobes. In megaloblastic anemia, segmentation is increased, and cells with six to even 10 or more nuclear lobes may be detected. Various measures have been proposed to quantitate the degree of hypersegmentation. The simplest screening technique is that of recording the number of segmented neutrophils with five or more lobes seen during the course of a 100-cell differential count. If more than three cells with five lobes, or even a single cell with six or more lobes, are observed, a presumptive diagnosis of hypersegmentation may be made. A "lobe average" may be determined by counting the number of lobes in the nuclei of 100 consecutive neutrophils and dividing by 100. Depending on the way in which a nuclear "lobe" is defined (see discussion in Chapter 6), the normal lobe average is  $3.17 \pm 0.25^{41}$  or  $2.03 \pm 0.39.^{54}$  An increase

in this value is one of the most sensitive indicators of the biochemical disorder that results in megaloblastic anemia.<sup>41</sup> Possibly even more sensitive is the calculation of the ratio of five-lobed to four-lobed granulocytes, which should be less than 0.17 in normal subjects.<sup>26</sup>

The main products of megaloblastic erythropoiesis are macrocytic erythrocytes with a distinctly oval shape. Such cells are well filled with hemoglobin and often the area of central pallor is reduced or absent. The oval shape may be particularly useful in distinguishing megaloblastic anemias from other causes of macrocytosis. The macroreticulocytes that characterize stimulated erythropoiesis tend to be round and distinctly blue or gray in Romanowsky stains. The thin macrocytes of liver disease usually are round, and, in addition, appear as target cells or as cells with increased areas of central pallor.

Although oval macrocytes tend to predominate, there may be considerable variation in the size (anisocytosis) (Fig. 14-4) and shape (poikilocytosis) of the erythrocytes. These changes are most marked when the anemia is pronounced. The most bizarre shapes may be seen: cells in the form of dumbbells, anvils, cocked bats, hand-mirrors, and so on. Many of these deformed erythrocytes are smaller in diameter than the cells of normal blood.

In patients with advanced anemia, a wide variety of red cell inclusions may be found. There may be many diffusely polychromatophilic cells and others that show punctate basophilia. The stippling may be fine or coarse. In occasional cells, Howell-Jolly bodies and acidophilic rings of various shapes (Cabot's rings) may be seen. In addition, orthochromic, polychromatophilic, or basophilic nucleated red cells may be found. The chromatin structure of the nuclei of these cells may be very fine so that they are readily recognized as megaloblasts (see below).

### Bone Marrow

The marrow is cellular and usually hyperplastic, with erythrocyte precursors predomi-

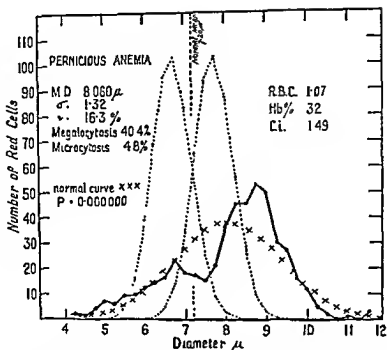


Fig 14-4 The variation in the diameters of the red corpuscles in a patient with pernicious anemia in relapse (continuous line) compared with the variation in health. The curves set out by the interrupted lines define the limits of normality as represented by  $\pm 3$  times the standard deviation of the mean diameter in the healthy adult. The degree of macrocytosis and microcytosis was estimated by counting the number of cells outside these boundaries. The skewness of the pernicious anemia curve is emphasized by comparison with the normal distribution curve (marked by crosses) for cells of this mean diameter. (From Price-Jones,<sup>40a</sup> courtesy of the author and Oxford University Press.)

nating. The characteristic *megaloblasts* are distinguished by their large size and, especially, by their delicate nuclear chromatin. The chromatin has been called "particulate" or "sieve-like," as distinguished from the normal denser chromatin of normoblasts, and resembles fine scroll work (Fig. 14-5, page 571, and Plate IX). This morphologic change may be detected at all stages of erythrocyte development. Thus, there are promegaloblasts and basophilic, polychromatophilic, and orthochromic megaloblasts. The cytoplasm of the very early stages is so deeply basophilic that it may be difficult to recognize these cells as erythrocyte precursors.

The identification of orthochromic megaloblasts is particularly useful in recognition of megaloblastic anemia because they differ so markedly from any cell found in normal marrow.<sup>41</sup> In the orthochromic megaloblast,

the abundant cytoplasm appears mature (pink), whereas the nucleus appears immature as the result of the megaloblastic change ("nuclear-cytoplasmic dissociation," "nuclear-cytoplasmic asynchrony"). An unusually large number of mitotic figures are to be found among the erythroid cells.

Associated with changes in the erythrocyte precursors is evidence of active and abnormal leukopoiesis. In addition to myeloid leukocytes of normal appearance, extraordinarily large (up to 20 or 30  $\mu$ m) leukocytes will be found. These monstrosities of cellular development may occur at any stage in the myeloid series, but they are particularly common among the metamyelocytes.<sup>33</sup> The nucleus of these so-called "giant metamyelocytes" is enlarged, both absolutely and in relation to the total cell size; it may be bizarre in shape and in chromatin structure or staining prop-

erties (Fig. 14-5). Occasionally small vacuoles are found in the cytoplasm. Giant metamyelocytes contain more than a diploid ( $2n$ ) amount of DNA and appear to be arrested in a premitotic stage of development.<sup>63</sup> It is therefore likely that they die within the marrow and are not precursors of the hypersegmented neutrophil.

In general, proliferation of megakaryocytes is less disturbed than that of either of the other two cell lines; however, when megaloblastic change is severe, megakaryocytes may

be reduced in number and there may be abnormalities of nuclear chromatin.<sup>30</sup> Furthermore, DNA synthesis may be arrested.<sup>57</sup>

All of the above changes vary in intensity depending on the degree of the deficiency or metabolic defect leading to the anemia. When deficiency is mild and there is little anemia, unequivocal megaloblasts in the marrow may be difficult to find. The terms "intermediate megaloblast" and "transitional megaloblast" have been used to describe the minimal alterations found in erythroid precursors under

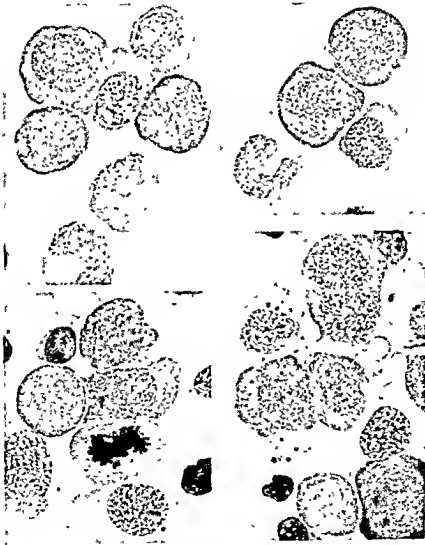


Fig. 14-5. Photomicrograph of the bone marrow of a patient with pernicious anemia in relapse. Megaloblasts in various stages of development are shown. Nucleoli are visible in some of these cells. The typical chromatin pattern should be noted. In the upper two photographs, "giant metamyelocytes" are also seen (Wright's stain  $\times 1040$ ).

such circumstances. This terminology has been criticized; possibly the phrase "early megaloblastic changes" is preferable.<sup>23</sup>

### *Erythrocyte Kinetics and the Metabolism of Iron and Bilirubin*

Erythrocyte survival in megaloblastic anemia generally is considered to be moderately reduced, although relatively few direct measurements have been made. In a total of five patients with pernicious anemia studied by the Ashby technique,<sup>50,59</sup> the survival of erythrocytes ranged from 27 to 75 days (normal, 120 days). Chromium half-disappearance times ( $T_{1/2}$  Cr) were found to be 16 to 21 days (normal, about 32 days) in three patients.<sup>11,43</sup> For the most part the excessive red cell destruction appears to result from a defect in the red cell itself. However, there also is evidence that normal cells transfused into patients with pernicious anemia are destroyed randomly at an increased rate.<sup>36</sup> The extracorporeal hemolytic factor suggested by these studies could be demonstrated *in vitro* in plasma, but it was not characterized further. It disappeared between three and nine days after vitamin B<sub>12</sub> was given. That at least part of the erythrocyte destruction occurs intravascularly is suggested by the observation that, in serum, haptoglobins are absent and methemalbumin may be present.<sup>83,95</sup>

The serum iron concentration is moderately increased unless there is complicating iron deficiency.<sup>23</sup> Bone marrow sideroblasts and reticuloendothelial iron stores also tend to be increased. The plasma total iron-binding capacity may be slightly reduced. Ferrokinetic studies (Table 14-2) have demonstrated a threefold increase in the plasma iron-transport rate. However, red cell utilization is markedly reduced, so that erythrocyte iron turnover is about normal. These kinetic data reflect ineffective erythropoiesis (Chapter 13).<sup>31,52</sup> The measures of "total" erythropoiesis, such as the plasma iron-transport rate and the degree of red cell hyperplasia, are increased. In contrast, measures of "effective" erythropoiesis, such as the erythrocyte iron-

**Table 14-2. Ferrokinetic Studies in Megaloblastic Anemia<sup>32</sup>**

	Normal Subjects	Patients with Pernicious Anemia
Plasma Fe ( $\mu$ g/dl)	105	152
Iron clearance ( $T_{1/2}$ minutes)	86	46
Plasma iron transport (mg/day/dl)	0.7	2.4
Red cell iron utilization (%)	80	28
Erythrocyte iron turnover (mg/day/dl)	0.56	0.62

Values are means of measurements in 107 normal subjects and in 27 patients with pernicious anemia.

turnover rate and the reticulocyte count, are not increased.

Additional evidence for ineffective erythropoiesis has been derived from the study of bile-pigment production. The daily excretion of urobilinogen greatly exceeds that which can be accounted for on the basis of destruction of circulating red cells.<sup>31</sup> Furthermore, there is a dramatic increase in the "early-labeled" fraction of bile pigments<sup>49</sup> (Chapter 5). These observations indicate that the probable source of the excessive bile pigment in megaloblastic anemia is destruction of erythrocyte precursors within the bone marrow.

As a result of the increased production of bile pigment, slight unconjugated ("indirect") hyperbilirubinemia may be observed, although normal values are common and values exceeding 2.0 mg/dl are unusual. Of 20 patients with pernicious anemia reported by Schilling and Harris the serum bilirubin was less than 1.0 mg/dl in 11, 1.0 to 1.5 in eight, and 2.2 in one.<sup>58</sup> These values were compared with previous reports indicating that 103 of a total of 126 patients had increased serum bilirubin concentrations. Schilling and Harris suggested that, as the result of earlier diagnosis and the availability of effective treatment, less severe disturbances in bilirubin metabolism were seen than had previously been observed.

### Serum Lactate Dehydrogenase and Other Enzymes

Lactate dehydrogenase (LDH) activity tends to be greatly increased in patients with megaloblastic anemia. In one series of 16 patients, the mean value was 3800 units/ml (normal mean 260 u/ml).<sup>21</sup> Values exceeding 10,000 u/ml have been reported. The magnitude of the increase is related to the degree of anemia; a linear relation between the logarithm of serum LDH activity and hemoglobin concentration was found in one series.<sup>29</sup> Serum LDH also may be increased in hemolytic anemia, but usually to a lesser degree than in megaloblastic anemia (Fig. 14-6).

Of the five common isoenzymes of LDH, the two which migrate fastest on electrophoresis (LDH-1 and LDH-2) are the ones that account for the increase in megaloblastic anemia.<sup>64</sup> LDH-1 activity exceeds that of LDH-2, whereas the converse is true in hemolytic anemia. Accurate electrophoretic isoenzyme quantitation may not always be available, but a relatively simple method based on chloroform inhibition can also distinguish between the pattern in hemolytic anemia and that in megaloblastic anemia.<sup>64</sup> In megaloblastic anemia, the source of the serum enzyme is thought to be the cells destroyed by ineffective erythropoiesis in the marrow. With specific therapy, serum LDH levels return to normal within one to two weeks.

A variety of other red cell enzymes may be found in the serum in increased amounts.<sup>28,39</sup> Malate dehydrogenase, 6-phosphogluconate dehydrogenase, and  $\alpha$ -hydroxybutyrate dehydrogenase are increased to about the same extent as LDH; aldolase, phosphohexose isomerase, and isocitrate dehydrogenase are increased less consistently and to a lesser degree. Serum alkaline phosphatase activity is decreased in patients with pernicious anemia<sup>25</sup> as is serum and red cell cholinesterase.<sup>51</sup>

### Changes in Epithelium

In a variety of epithelial tissues, morphologic alterations that somewhat resemble the

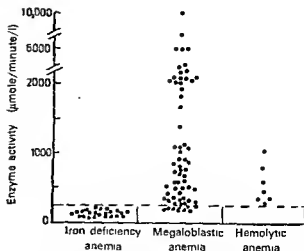


Fig. 14-6 Serum lactate dehydrogenase activity in megaloblastic anemia, compared with iron-deficiency and hemolytic anemias. The dashed line indicates the upper limit of normal. (From Emerson and Wilkinson,<sup>29</sup> courtesy of the authors and Blackwell Scientific Publications.)

changes in marrow red cell precursors may be observed. The cells and their nuclei are abnormally large, nuclear chromatin is less coarse than normal, and multiple nuclei may be found. Such abnormalities may be in cells from the buccal,<sup>23</sup> gastric,<sup>34</sup> and vaginal mucosae<sup>34,48</sup> (Fig. 15-6, page 617).

### Cytogenetics

A variety of chromosomal abnormalities have been described in marrow cells from patients with megaloblastic anemia.<sup>37,44,45,46</sup> Individual chromosomes may be elongated and there is an increased frequency of randomly distributed gaps and breaks. Separation or spreading of the centromere has been observed.<sup>37</sup> Most investigators have reported a normal diploid number of chromosomes, but some have observed hypodiploidy.<sup>45</sup> The chromosomal abnormalities disappear promptly with effective treatment.<sup>44,46</sup>

### Miscellaneous Biochemical Findings

The serum potassium concentration may be slightly reduced; in one study, the values in 17 of 34 patients were less than 4.0 mEq/l, the lower limit of normal in that laboratory.<sup>47</sup>



Negative nitrogen balance is usually observed during relapse.<sup>42</sup> There may be slight excessive urinary excretion of taurine and other amino acids.<sup>33,62</sup> An increase in urinary hydroxyphenyl compounds, probably derived from tyrosine, has been observed.<sup>61</sup> Conversion of formate to serine by lymphocytes is inhibited.<sup>27</sup>

### Disorders Associated with Megaloblastic Anemia

In the great majority of patients, megaloblastic anemia is a consequence of deficiency of vitamin B<sub>12</sub> or folate, or both (Table 14-3). Nutritional aspects and the metabolism of these vitamins as well as their role in DNA

synthesis were discussed in Chapter 4. Rare causes of megaloblastic anemia include congenital disorders affecting DNA synthesis and certain drug-induced disorders (Table 14-3).

### Dietary Deficiency of Vitamin B<sub>12</sub>

Dietary deficiency of vitamin B<sub>12</sub> is very unusual because of the ubiquitous distribution of this vitamin and the extensive hepatic stores. Only strict vegetarians who avoid not only meat but also eggs and milk ("vegans") have been observed to develop vitamin B<sub>12</sub> deficiency because of dietary inadequacy.<sup>119,142</sup> Even in vegans, megaloblastic anemia is unusual; in one study, biochemical

**Table 14-3. Pathogenetic Classification of the Causes of Megaloblastic Anemia**

I Vitamin B <sub>12</sub> deficiency	1 Cirrhosis
A Dietary deficiency (rare)	2 Pregnancy
B Lack of Castle's intrinsic factor	3 Infancy
1 Pernicious anemia	4 Diseases associated with rapid cellular proliferation
a Congenital form	C Congenital folate malabsorption
b Adult form	D Drug-induced folate malabsorption
2 Gastrectomy	1 Anticonvulsants
a Total	2 Oral contraceptives
b Partial	E Extensive intestinal resection, jejunal resection
3 Ingestion of caustic materials	III Combined folate and vitamin B <sub>12</sub> deficiency
C Functionally abnormal intrinsic factor	A Tropical sprue
D Biologic competition	B Gluten sensitive enteropathy
1 Small-bowel bacterial overgrowth	IV Inherited disorders of DNA synthesis
a Small bowel diverticulosis	A Orotic aciduria
b Anastomoses and fistulae	B Lesch-Nyhan syndrome
c Blind loops and pouches	C Thiamin-responsive megaloblastic anemia
d Strictures	D Deficiency of enzymes required for folate metabolism
e Scleroderma	1 N <sup>5</sup> -methyl tetrahydrofolate transferase
f Achlorhydria	2 Formiminotransferase
2 Fish tapeworm disease	3 Dihydrofolate reductase
E Familial selective vitamin B <sub>12</sub> malabsorption (Imerslund's syndrome)	E Congenital megaloblastic anemia responsive to large doses of folate and vitamin B <sub>12</sub>
F Drug-induced vitamin B <sub>12</sub> malabsorption	V Drug induced disorders of DNA synthesis
G Chronic disease of the pancreas	A Folate antagonists (eg. methotrexate)
H Zollinger-Ellison syndrome	B Purine antagonists (eg. 6-mercaptopurine)
I Diseases especially affecting the ileum	C Pyrimidine antagonists (eg. cytosine arabinoside)
1 Ileal resection and bypass	VI Erythroleukemia
2 Regional enteritis	
II Folate deficiency	
A Dietary deficiency	
B Increased requirements	

evidence of vitamin B<sub>12</sub> deficiency was observed in only three of 26 vegans, none of whom was anemic.<sup>112</sup>

It follows that deficiency of vitamin B<sub>12</sub> is nearly always the result of defective absorption of the vitamin. This may occur because of lack of Castle's intrinsic factor (Chapter 4) or because of various disorders affecting the small intestine (Table 14-3).

### ***Pernicious Anemia***

The most common cause of intrinsic factor deficiency is pernicious anemia, which is discussed in Chapter 15.

### ***Gastrectomy***

Total gastrectomy removes all intrinsic factor-secreting cells, and vitamin B<sub>12</sub> deficiency will inevitably supervene if sufficient time elapses without parenteral vitamin therapy. The average duration from the time total gastrectomy was performed to onset of anemia was found to be five years (range two to 10 years).<sup>23</sup>

Megaloblastic anemia may also follow partial gastrectomy, even though this operation leaves intact some of the intrinsic factor-secreting parietal cells. Malabsorption of vitamin B<sub>12</sub> has been found in approximately 30 to 40% of subtotal gastrectomy subjects.<sup>117,129</sup> The absorptive defect is usually less severe than that occurring in pernicious anemia or after total gastrectomy.<sup>108,132</sup> Nevertheless, when follow-up was prolonged the incidence of megaloblastic anemia was as great as 18%.<sup>120</sup> The increased incidence with time following the operation<sup>141</sup> may possibly be due to gastritis developing in the gastric remnant.<sup>127,135</sup> Defective vitamin B<sub>12</sub> absorption was more common when the operation was performed for gastric ulcer than for duodenal ulcer.<sup>124,129</sup> Iron-deficiency anemia often complicates the picture and may obscure the megaloblastic changes. Although vitamin B<sub>12</sub> deficiency accounts for about 80% of the cases of megaloblastic anemia

following subtotal gastrectomy, folate deficiency also has been observed.<sup>120,133</sup> The pathogenesis of the latter is unknown.

### ***Ingested Corrosive Materials***

Megaloblastic anemia following destruction of gastric mucosa by ingested corrosive materials has rarely been reported.<sup>101</sup>

### ***Functionally Abnormal Intrinsic Factor***

In a report of a single patient, vitamin B<sub>12</sub> deficiency appeared to result from secretion of biologically inert intrinsic factor.<sup>125</sup> Intrinsic factor obtained from this patient was normal with respect to immunologic reactivity, chromatographic properties, and vitamin B<sub>12</sub> affinity; however, it could not correct the vitamin B<sub>12</sub> malabsorption of a gastrectomized subject nor could it stimulate vitamin B<sub>12</sub> uptake by homogenates of guinea pig ileum. The patient responded completely to parenteral administration of vitamin B<sub>12</sub>.

### ***Small Bowel Bacterial Overgrowth***<sup>111,114</sup>

The proximal small bowel in healthy subjects may be sterile or it may contain relatively low concentrations (usually less than 10<sup>3</sup> organisms/ml of contents) of gram-positive aerobes or facultative anaerobes, such as lactobacilli and enterococci along with some coliforms.<sup>114</sup> Normally, bacterial overgrowth in the small intestine is prevented to some extent by gastric acid secretion; however, the most important factor appears to be the mechanical cleansing action of normal peristalsis.<sup>111</sup> Any anatomic abnormality leading to stasis or recirculation of intestinal contents is likely to be accompanied by proliferation of microorganisms. The most common lesions that may have this effect are listed in Table 14-3 (I, D, 1, a-f). The bacterial population developing under such circumstances resembles that of the colon. Anaerobic lactobacilli and bacterioides tend to predominate, and coliforms and clostridia

may be found in high concentrations. The numbers of organisms may be large, up to  $10^{10}$ /ml.

The principal manifestations of the bacterial overgrowth syndrome are weight loss, megaloblastic anemia, steatorrhea, and diarrhea. The steatorrhea probably results from bacterial hydrolysis of conjugated bile salts to bile acids, which then are reabsorbed. By this mechanism, the concentration of intestinal bile salts becomes reduced below the critical level necessary for micelle formation, leading to malabsorption of fatty acids and monoglycerides.<sup>114,126</sup>

The megaloblastic anemia almost always results from vitamin B<sub>12</sub> deficiency. With rare exceptions,<sup>102</sup> folate absorption is not impaired; body folate stores are normal and may even be increased because of absorption of microbially synthesized folate.<sup>121</sup> Vitamin B<sub>12</sub> absorption is uniformly reduced in the bacterial overgrowth syndromes; the defect is not corrected by administration of intrinsic factor, but is transiently corrected by prior administration of certain antibiotics, especially tetracyclines.<sup>116,132</sup> Most probably, the absorptive defect represents successful competition by the bacteria for dietary vitamin B<sub>12</sub>. The bacteria appear to be able to take up and bind the vitamin so firmly that it is essentially unavailable to the host.<sup>106,109</sup> The amount of the vitamin bound greatly exceeds the needs of the bacteria.<sup>114</sup> Surgical correction of the anatomic lesion of the bowel leads to normalization of the bowel flora and restoration of normal vitamin B<sub>12</sub> absorption.<sup>111</sup>

### Fish Tapeworm Disease

In Finland, megaloblastic anemia due to vitamin B<sub>12</sub> deficiency has been observed in 1.9 to 3.0% of carriers of the fish tapeworm, *Diphyllobothrium latum*.<sup>134</sup> *D. latum*, a common parasite of freshwater fish, especially pike, is widely distributed in the lakes of many parts of Europe as well as those of north central North America. Human infection results from ingestion of inadequately cooked fish. Despite the wide distribution of

the parasite, it rarely, if ever, causes megaloblastic anemia outside of Finland. The reason for the precise geographic distribution is not clear; that a constitutional predisposition is required before anemia develops has been suggested.<sup>103</sup> More likely, the development of deficiency depends on the mass of worms harbored by the patient.<sup>115</sup> This in turn may be related to the dietary habits of the Finns or to special characteristics of the variety of *D. latum* in Finland.

The deficiency of vitamin B<sub>12</sub> results from impaired absorption. Little, if any, improvement in absorption was observed when the vitamin was given with intrinsic factor.<sup>134</sup> As in the bacterial overgrowth syndromes, the malabsorption probably results from competition between the worm and the host for dietary vitamin B<sub>12</sub>. The tapeworm is able to take up and bind the vitamin firmly.<sup>134</sup> It lodges in the ileum in carriers without anemia, whereas in those with anemia the site of attachment tends to be in the jejunum.<sup>104</sup> Apparently, this latter location enables the worm to bind the vitamin before it reaches the sites of absorption in the ileum.

The anemia responds to expulsion of the worms,<sup>134</sup> but the response often is suboptimal if no vitamin B<sub>12</sub> is given.<sup>104</sup> Vitamin B<sub>12</sub> is effective even when the worms are not expelled.

### Familial Selective Vitamin B<sub>12</sub> Malabsorption (Imerslund's Syndrome)<sup>122</sup>

This inherited illness is characterized by the onset of megaloblastic anemia in childhood, usually during the first two years of life, and by persistent proteinuria.<sup>131</sup> The disease is rare, only 38 cases having been reported.<sup>170,131,139</sup> It appears to be inherited as an autosomal recessive trait.

Patients with this disorder are unable to absorb vitamin B<sub>12</sub> whether or not it is bound to intrinsic factor. Except in one patient,<sup>139</sup> other abnormalities of intestinal absorption have not been observed. Furthermore, there is no defect in gastric secretion, nor are gas-

tric autoantibodies (page 606) found. The anemia responds completely to vitamin B<sub>12</sub>, but the proteinuria persists. In heterozygotes, defects in vitamin B<sub>12</sub> absorption lesser in severity than in homozygotes have been reported, but anemia does not develop.<sup>131</sup>

The nature of the inherited lesion remains unknown. It has been suggested that ileal receptors for vitamin B<sub>12</sub> are defective,<sup>131</sup> but in at least one well-studied patient, ileal homogenates bound the vitamin normally, indicating that the receptor sites were intact.<sup>130</sup> This observation, together with the absence of morphologic lesions as seen by light or electron microscopy of ileal biopsy specimens, suggests that the inherited lesion impairs a stage of vitamin B<sub>12</sub> absorption that follows ileal attachment of the intrinsic factor-B<sub>12</sub> complex.

#### ***Drug-Induced Vitamin B<sub>12</sub> Malabsorption***

Megaloblastic anemia is a rare effect of para-aminosalicylic acid (PAS) therapy, having been observed in only two subjects receiving the drug for treatment of tuberculosis.<sup>118</sup> A moderate degree of vitamin B<sub>12</sub> malabsorption is found, however, in nearly all subjects who consume PAS for more than six weeks. The defect appears to be "selective," in that fat and xylose absorption are unaffected. Intrinsic factor secretion is not impaired. The absorptive defect disappears about two weeks after use of the drug is discontinued. Reversible malabsorption of vitamin B<sub>12</sub> has also been observed in patients taking colchicine,<sup>140</sup> neomycin,<sup>123</sup> ethanol,<sup>128</sup> or potassium chloride.<sup>136</sup>

#### ***Chronic Pancreatic Disease***

Defective absorption of vitamin B<sub>12</sub>, correctable with oral administration of pancreatic enzyme, was detected in nine of 22 patients with pancreatic exocrine insufficiency.<sup>138</sup> Megaloblastic anemia has been observed only rarely in association with this disorder, however, possibly because the de-

fect usually is not severe enough nor of sufficient duration for deficiency to develop. A similar abnormality can be induced in rats by partial pancreatectomy.<sup>138</sup>

#### ***Zollinger-Ellison Syndrome***

Impaired vitamin B<sub>12</sub> absorption without megaloblastic anemia was observed in patients with Zollinger-Ellison syndrome, probably because of the low pH of intestinal contents reaching the ileum.<sup>137</sup>

#### ***Diseases Affecting the Ileum***

Since vitamin B<sub>12</sub> is absorbed chiefly in the ileum, ileal resection or bypass or diseases that may be localized to the ileum lead to malabsorption of the vitamin. Vitamin B<sub>12</sub> malabsorption has been observed in patients who have had as little as 1 foot of ileum resected, and probably is always impaired if more than 6 feet are removed.<sup>105,113</sup> Absorption of the vitamin was uniformly impaired in six patients after they had had ileal bypass operations.<sup>107</sup> In contrast, resection of the jejunum does not usually affect vitamin B<sub>12</sub> absorption, but may impair folate absorption.

The terminal ileum is affected in about 80% of patients with regional enteritis (Crohn's disease), and about 18% of such patients have been found to have megaloblastic anemia.<sup>23</sup> Both folate deficiency and vitamin B<sub>12</sub> deficiency may occur in association with regional enteritis, but there are no good data indicating relative frequency of the two deficiencies.

#### ***Dietary Folate Deficiency***

That a short period of dietary deprivation can lead to folate deficiency in an otherwise healthy individual has been demonstrated convincingly.<sup>40</sup> Normal body folate stores remain adequate for only two to four months following the institution of a deficient diet, and in this respect folate differs markedly from vitamin B<sub>12</sub>, the stores of which can last for several years.

Nutritional folate deficiency is especially common in tropical areas such as India,<sup>246,263</sup> Burma,<sup>224</sup> Singapore,<sup>253</sup> Malaysia,<sup>258</sup> and Africa.<sup>201, 214, 260</sup> In these areas the inadequate diet is chiefly a consequence of extreme poverty, but ignorance, customs, and religious tenets may also affect intake adversely. The diets associated with the development of folate deficiency are characterized by a pre-dominance of starches and grains with relatively little animal protein or fresh, green vegetables. In a number of these areas, tropical sprue (see below) is endemic, and the relative importance of this illness and nutritional factors has not been easy to assess.<sup>23</sup> When vitamin B<sub>12</sub> deficiency is associated with "tropical macrocytic anemia,"<sup>204</sup> it is usually considered to be caused by tropical sprue.

Nutritional folate deficiency has also been found in relatively affluent populations living in temperate areas.<sup>211, 213, 217, 218, 243, 261</sup> Major factors contributing to the consumption of poor diets in such people were mental disturbances, chronic illness, alcoholism, old age, food fadism, and poverty. Dietary folate deficiency may occur more often in Great Britain than in the United States because of habitual use, in Great Britain, of vegetable cooking methods utilizing long exposure to heat and water.<sup>220</sup>

### *Cirrhosis*

Megaloblastic anemia has been observed in about 20% of patients with alcoholic cirrhosis.<sup>2, 7, 226, 227, 232</sup> In some of the subjects the marrow has been said to have "transitional" megaloblastic changes, perhaps because the morphologic characteristics have been modified by coexisting iron deficiency.<sup>227</sup> The incidence of megaloblastic anemia in association with cirrhosis may be as great as 50% or more if the diagnosis is made on the basis of such early signs as macroovalocytosis of the red cells and hypersegmentation of the granulocytes.<sup>219</sup> In general, the patients with megaloblastic anemia tend to have more se-

vere degrees of anemia and more pronounced macrocytosis (MCV greater than 120 fl) than do patients with non-megaloblastic macrocytic anemia associated with liver disease (Chapter 19, page 706).<sup>2, 226</sup>

Megaloblastic anemia in patients with cirrhosis almost always is the consequence of folate deficiency. Serum folate levels are reduced,<sup>219, 226, 230, 243</sup> formiminoglutamate (FIGlu) excretion (page 586) is increased,<sup>207</sup> and vitamin B<sub>12</sub> levels are normal or increased.<sup>227, 243</sup> The folate deficiency results chiefly from inadequate dietary intake.<sup>219, 230</sup> However, there also is evidence of disordered folate metabolism, as manifested by decreased ability of the liver to store folate<sup>208</sup> and excessive urinary loss of the vitamin.<sup>247</sup> Because of this, there is an increased need for dietary folate in patients with liver disease.

### *Pregnancy*

The megaloblastic anemia of pregnancy probably is the most common of all folate deficiency states.<sup>23</sup> It has been discussed in Chapter 13 in relation to other forms of anemia occurring during pregnancy.

### *Infancy*

In 1946, Zuelzer and associates called attention to the occurrence in infants (age two to 16 months) of folate-deficiency megaloblastic anemia resulting from increased needs, dietary inadequacy, and superimposed infection.<sup>265</sup> Within the next few years, their observation was followed by reports of over 100 cases. In about a quarter of the reported patients, evidence of coexisting scurvy was found. There was a rapid decrease in the number of reported cases after vitamin C was added to infant feeding formulas.<sup>234, 237, 239, 263</sup> These observations led to speculation that vitamin C was required in some way for normal folate metabolism.<sup>242</sup> No evidence supporting this speculation was forthcoming, and in retrospect it is likely that the affected infants had a simultaneous de-

iciency of vitamin C and folate. Vitamin C added to the feeding formulas probably acts as a preservative for their intrinsic folate content, especially when the formulas are heated during preparation.

Megaloblastic anemia is now uncommon among infants in well-developed, affluent countries. It continues to be observed in areas where malnutrition is widespread,<sup>235,239,262</sup> for example, those in which kwashiorkor is endemic.<sup>23,235</sup>

The normal requirement for folate in infancy has been estimated to be about 50  $\mu\text{g}/\text{day}$ , or about four to ten times the adult requirement on a weight basis.<sup>257</sup> Normal human milk and raw or pasteurized cow's milk contain about 50  $\mu\text{g}$  of folate per liter.<sup>216,241</sup> Thus, with normal rates of milk intake, the infant requirements for folate can be satisfied with milk alone. However, boiling leads to a 40 to 80% reduction in the folate content of milk, and folate is partially destroyed in preparing powdered milk. In general, serum folate levels are higher in breast-fed than in bottle-fed infants.<sup>240</sup> Nevertheless, megaloblastic anemia has been reported in breast-fed infants,<sup>203</sup> possibly because of decreased amounts of folate in the milk of mothers with marginal folate stores.<sup>256</sup>

Goat's milk contains very low amounts of folate (average, 6  $\mu\text{g}/\text{l}$ ).<sup>216,241</sup> "Goat's milk anemia" is a form of megaloblastic anemia of infancy occurring in babies fed goat's milk exclusively. First described in Germany and Italy, it has also been observed in the United States,<sup>257,265</sup> Australia,<sup>264</sup> and New Zealand.<sup>203</sup>

Nutritional folate deficiency in infants must be distinguished from certain other disorders causing megaloblastic anemia in this age group. These disorders include congenital pernicious anemia (Chapter 15), familial selective vitamin  $\text{B}_{12}$  malabsorption (page 576), celiac disease (page 580), and inborn errors of DNA synthesis (page 581). Also, vitamin  $\text{B}_{12}$  deficiency has been observed in the breast-fed infant of a mother with subclinical pernicious anemia.<sup>233</sup>

### *Rapid Cellular Proliferation*

Folate deficiency with megaloblastosis has been observed as a complication of a variety of hematologic illnesses. These include sickle cell anemia,<sup>202,236</sup> thalassemia,<sup>225,250</sup> hereditary spherocytosis,<sup>210,231</sup> acquired autoimmune hemolytic anemia,<sup>212</sup> drug-induced hemolytic anemia with glucose-6-phosphate dehydrogenase deficiency,<sup>245</sup> paroxysmal nocturnal hemoglobinuria,<sup>244</sup> myelofibrosis,<sup>222</sup> sideroblastic anemia,<sup>223,238</sup> leukemia,<sup>251</sup> and multiple myeloma.<sup>221</sup> The complication is associated with a fall in the VPRC from previously stable levels or with an increase in transfusion requirements.

These disorders have in common a rapid proliferation of red cells or other marrow elements. It is reasonable to assume that the need for DNA synthesis, and therefore the need for folate, is increased under such circumstances. Often, however, there have been other coexisting reasons for folate deficiency in the reported patients, including pregnancy and deficient diet.

### *Congenital Folate Malabsorption*

Congenital folate malabsorption is an extremely rare disorder, having been reported in only three patients in two different families.<sup>234</sup> In addition to the absorptive disorder, defective transfer of folate to the central nervous system associated with mental retardation and ataxia was observed.

### *Drug-Induced Folate Malabsorption*

Severe megaloblastic anemia has been observed in patients taking anticonvulsant drugs.<sup>228,249,254</sup> The drugs implicated in the majority of these patients were diphenylhydantoin (Dilantin), primidone (Mysoline), and phenobarbital, alone or in combination. Although well-marked megaloblastic anemia was unusual, evidence of folate deficiency and mild hematologic changes were relatively common. For example, serum folate levels were reduced in 31 to 76% of unselected

patients receiving anticonvulsants.<sup>23,209,229</sup>  
<sup>259</sup> In one series, serum folate values were subnormal in 76% and megaloblastosis was found in 38% of 45 nonanemic patients.<sup>219</sup>

Anticonvulsant drugs appear to exert their effects by impairing folate absorption.<sup>215</sup> This abnormality may result from inhibition of intestinal conjugase with consequent failure to absorb folic polyglutamates.<sup>252</sup> However, in one study, no *in vitro* evidence for conjugase inhibition could be found, and it was suggested that it is absorption of free folate that is defective.<sup>206</sup> This controversy will probably be resolved in the near future when labeled folic polyglutamates and monoglutamates become available for absorption tests.

A similar defect in folate absorption with associated megaloblastic anemia has been reported in women taking oral contraceptive medications.<sup>255,259</sup> When oral tolerance tests were performed in a nondeficient group of women taking contraceptives, absorption of folate monoglutamate appeared to be normal, but folate polyglutamate absorption was impaired.<sup>251</sup>

### *Tropical Sprue and Gluten-Sensitive Enteropathy*

Tropical sprue and gluten-sensitive enteropathy are similar in that both bring about a generalized state of intestinal malabsorption characterized by steatorrhea, weight loss, weakness, and deficiency of a wide variety of nutrients. The morbid anatomy in both is characterized by varying degrees of villous atrophy with loss of intestinal surface. In gluten-sensitive enteropathy, the lesions are most severe in the proximal intestine, and the ileum is involved in only a minority of the patients.<sup>281</sup> In tropical sprue, the lesions tend to be less severe but more extensive, the whole of the small intestine being affected.<sup>279</sup> The diseases differ not only in their geographic distribution, but also in pathogenesis and response to specific modes of therapy.

*Tropical sprue*<sup>279</sup> is endemic in most of the West Indies, South India, Southeast Asia, Malaysia, and Indonesia; it is rare, however,

in certain other tropical areas, such as Jamaica, Africa, and South America. There have been reports of patients who developed the illness months to years after leaving an endemic area. Although the cause of the illness is unknown, an infectious origin seems probable on the basis of observed responses to antibiotic therapy<sup>277,279</sup> and on epidemiologic grounds.<sup>271</sup> A specific infectious agent has not been identified with certainty, but preliminary evidence suggests that it might be the zygote form of the alga, *Prototheca portoricensis*.<sup>272</sup> If this cause can be established, tropical sprue would be the first known algal disease in man.

Three clinical stages of tropical sprue have been recognized.<sup>276,285</sup> The initial phase is characterized by the abrupt onset of diarrhea, anorexia, abdominal distension, and extreme asthenia. After an interval of weeks to months, the second, or "deficiency" phase occurs as the result of prolonged malabsorption and depletion of nutrients. Weight loss, glossitis, stomatitis, hyperkeratosis, and continuing weakness are prominent in this stage. In the third phase, megaloblastic anemia develops. Since the intestinal lesion tends to be generalized, absorption of both folate and vitamin B<sub>12</sub> is impaired. In disease of relatively short duration, folate deficiency predominates, whereas in the more chronic forms of the illness, vitamin B<sub>12</sub> deficiency occurs as well.<sup>23</sup> The described sequence of events is not invariable, and patients with megaloblastic anemia may be observed who have not experienced the other clinical manifestations of tropical sprue.

Administration of folate to patients with tropical sprue not only induces hematologic remission, but also may bring about improvement in intestinal symptoms and pathologic lesions.<sup>286</sup> In about 50% of patients, however, intestinal dysfunction may persist after folate therapy, and may later respond to antibiotics.<sup>277</sup>

*Gluten-induced enteropathy* is a term that includes both celiac disease of children and nontropical sprue (idiopathic steatorrhea) in adults. The illness represents a possibly inherited,<sup>280,281</sup> abnormal reaction to gluten, a

water-insoluble, glutamine-rich protein fraction of wheat and other grains. The mechanism of gluten sensitivity is not understood; it has been suggested that the patients lack an intestinal peptidase that normally acts to detoxify a toxic peptide in gluten.<sup>270</sup>

In addition to weight loss, abdominal distension, and steatorrhea, patients with gluten-induced enteropathy may develop negative calcium balance with hypocalcemia, tetany, and demineralization of bones, with consequent bone pain and pathologic fractures. They may also develop deficiency of vitamin K-dependent coagulation factors (Chapter 38).

In celiac disease in children, the most common cause of anemia is iron deficiency,<sup>274</sup> but folate deficiency occurs in 10 to 40%. Vitamin B<sub>12</sub> deficiency is unusual. In the adult form of the disease, folate malabsorption and deficiency are found in about 90% of patients, whereas vitamin B<sub>12</sub> malabsorption and deficiency are found in only about 40%,<sup>23</sup> presumably because the ileum often is spared.<sup>253</sup> Iron stores often are reduced, but the anemia rarely appears hypochromic until the megaloblastic defect has been corrected. To obtain an optimal hematologic response to folate or vitamin B<sub>12</sub>, concurrent or subsequent iron therapy may be required. With a gluten-free diet the blood returns to normal over a period of several months.<sup>275</sup>

In occasional patients, folate malabsorption seems out of proportion to the other intestinal abnormalities. Such patients manifest megaloblastic anemia without other clinical evidence of malabsorption ("temperate sprue").<sup>273</sup> Whether this represents a unique illness or a variant of nontropical sprue is uncertain.

Certain abnormalities are demonstrable by laboratory tests in both tropical sprue and gluten-induced enteropathy. Total stool fat is increased<sup>282</sup> and D-xylose absorption is defective.<sup>284</sup> Serum carotene and vitamin A levels usually are reduced.<sup>288</sup> A "malabsorption pattern" is observed on roentgenographic study of the intestine<sup>287</sup>; the jejunum is dilated, the barium column becomes bro-

ken up into segments, the valvulae conniventes may be obliterated or widely separated, and the bowel segments may resemble "a tube into which wax has been poured and allowed to harden" (moulage sign)<sup>278</sup> (Fig. 14-7). Subtotal villous atrophy is found on jejunal biopsy.<sup>279,283</sup> The diagnosis of gluten sensitivity usually requires that a response to a gluten-free diet be demonstrated. Alternatively, when the diagnosis is difficult to establish, the patient may be challenged with an oral dose of 30 to 50 g of gluten which, in sensitive subjects, is followed by a prompt increase in diarrhea and fecal fat loss.

When the manifestations of tropical sprue and gluten-induced enteropathy are tabulated and compared with those in pernicious anemia, it becomes evident that there are certain clinical similarities in the illnesses, but that certain manifestations tend to predominate in each (Table 14-4). Cases of pernicious anemia are encountered in which glossitis and diarrhea are so prominent that differentiation from sprue is difficult; conversely, when the intestinal manifestations of sprue are not prominent, the disease may appear similar to pernicious anemia.

### *Inherited Disorders Affecting DNA Synthesis*

**OROTIC ACIDURIA.** This is an inherited disorder of pyrimidine metabolism characterized by megaloblastic anemia and excessive urinary excretion of orotic acid.<sup>301</sup> In most of the patients reported, growth and development were retarded. The illness probably is transmitted as an autosomal recessive trait, and is very rare, having been reported in only seven patients.<sup>296,299,301,303</sup> In six of the seven, the disease resulted from greatly reduced activity of two enzymes, orotidylic pyrophosphorylase and orotidylic decarboxylase, both of which are required for the conversion of orotic acid to uridine-5-phosphate. In the seventh patient, the activity of only one of the two enzymes (orotidylic decarboxylase) was reduced.<sup>296</sup> In heterozygotes, the activity of the two enzymes may be somewhat reduced, and slightly increased





Fig 14.7 Roentgenogram showing the gastrointestinal tract two hours following a barium meal in a patient with sprue. The stomach is almost completely empty. The barium is distributed throughout the small intestine and has reached as far as the splenic flexure of the large intestine. The small bowel shows the characteristic 'moulage' sign. The pattern of an irritative lesion in the jejunum is shown by the many small irregular collections of barium on the left side of the abdomen.

values for urinary orotic acid may be found, but no disease results.<sup>295</sup> Orotic aciduria does not respond to folate or vitamin B<sub>12</sub>, but administration of uridine (1 to 1.5 g/day) leads to hematologic remission and restoration of normal growth and development.

**LESCH-NYHAN SYNDROME.** This is an X-linked disorder of purine metabolism, characterized by hyperuricemia, self-mutilation, and mental and neurologic defects. It results from a lack of the enzyme hypoxanthine-guanine phosphoribosyl-transferase. In one case, the syndrome included megaloblastic anemia which responded to the administration of adenine.<sup>306</sup>

**THIAMIN-RESPONSIVE MEGALOBlastic ANEMIA.** An 11 year old girl with severe megaloblastic anemia, diabetes mellitus, aminoaciduria, and sensorineural deafness was reported.<sup>300</sup> She failed to respond to folate, vitamin B<sub>12</sub>, uridine, or many other nutrients; however, on two occasions, a reticulocytosis and a rise to normal in the hemoglobin level were observed when thiamin was administered in a dose of 20 mg per day. The nature of the metabolic defect was not elucidated.

**N<sup>5</sup>-METHYL TETRAHYDROFOLATE TRANSFERASE DEFICIENCY.** A single case of congenital megaloblastic anemia and mental retardation

**Table 14-4. Comparison of Certain Manifestations of Pernicious Anemia, Tropical Sprue, and Gluten-Induced Enteropathy**

	Pernicious Anemia	Tropical Sprue	Gluten Enteropathy
Mental symptoms	+	-	-
Neurologic disease	++	+	-
Absence of intrinsic factor	+++	+	-
Macrocytic anemia	+++	++	+
Glossitis	++	+++	+
Diarrhea	+	+++	++
Weight loss	+	+++	+++
Steatorrhea	-	+++	+++
Hypocalcemia	-	+	+++
Hypochromic anemia	-	+	++
Bone deformities	-	-	+++

\*Especially in children

tion associated with very high serum folate levels has been reported.<sup>291</sup> Presumably the serum folate could not be utilized, since a reticulocyte response followed treatment with folic acid. In liver specimens, there was a severe decrease in activity of the enzyme catalyzing the conversion of N<sup>5</sup>-methyl tetrahydrofolate to tetrahydrofolate.

**FORMIMINOTRANSFERASE DEFICIENCY.** This inborn error of folate metabolism has been detected in four subjects. Three were hematologically normal, but one had "megaloblastic and sideroblastic" anemia.<sup>291</sup>

**DIHYDROFOLATE REDUCTASE DEFICIENCY.** In a patient with congenital megaloblastic anemia, dihydrofolate reductase activity in liver tissue was found to be markedly reduced.<sup>304</sup> The patient did not respond to folic acid but did respond to folinic acid (N<sup>5</sup>-formyltetrahydrofolic acid), a reduced form of the vitamin. It was concluded that the findings in this patient were best explained by a congenital lack of dihydrofolate reductase.

**TRANSCOBALAMIN II DEFICIENCY.** Two sisters developed growth failure and megaloblastic anemia at ages three and five weeks.<sup>297</sup> The vitamin B<sub>12</sub> transport protein, transcobalamin II, was lacking in the sera of both,

and this defect probably was inherited as an autosomal recessive trait. Both infants responded to high-dose (2000 µg/week) vitamin B<sub>12</sub> therapy, and in one of them relapse occurred six weeks after therapy was withdrawn.

**CONGENITAL MEGALOBlastic ANEMIA RESPONSIVE TO COMBINED, LARGE-DOSE FOLATE AND VITAMIN B<sub>12</sub> THERAPY.** The occurrence of congenital megaloblastic anemia in two sisters was reported.<sup>298</sup> Serum vitamin B<sub>12</sub> and serum erythrocyte folate levels were normal. Optimal hematologic response required the simultaneous administration of both folate and vitamin B<sub>12</sub> in large doses. The nature of the defect was not determined.

### *Drug-Induced Disorders of DNA Synthesis*

Megaloblastic anemia is a predictable toxic effect of a variety of drugs used in the chemotherapy of leukemia and solid tumors (Chapter 55). The complication has been reported with 6-mercaptopurine, 5-fluorouracil,<sup>294</sup> cyclophosphamide,<sup>293</sup> cytosine arabinoside,<sup>302</sup> and the folate antagonists.<sup>305</sup> In these situations, the toxic effect is an extension of the therapeutic effect. The drugs are used because they interfere with DNA synthesis and therefore inhibit growth of rapidly dividing tissues. When toxicity occurs, pancytopenia and hypersegmented neutrophils are found, as well as oval macrocytes in the blood and megaloblasts in the bone marrow.

The folate antagonists, of which amethopterin (methotrexate) is the most commonly used example, act chiefly by competitive inhibition of dihydrofolate reductase.<sup>305</sup> When this enzyme is inhibited, dietary folate cannot be converted to the active, tetrahydro form, and endogenous folate accumulates as the inactive dihydrofolate.

Inhibition of dihydrofolate reductase has been reported as a side effect of drugs used for other purposes. These drugs include pyrimethamine, triamterene, pentamidine, and trimethoprim.<sup>305</sup> They are much less potent antagonists than methotrexate, and are likely to produce megaloblastic anemia only in individuals with borderline folate stores.

### Erythroleukemia (Di Guglielmo Syndrome)

Megaloblasts, or cells resembling them, have been described in the blood and marrow of patients with neoplastic proliferation of red cell precursors<sup>292</sup> (see Chapter 48). In these subjects, the megaloblasts do not disappear after treatment with vitamin B<sub>12</sub> or folate, and serum levels of these vitamins are either normal or increased. This syndrome must be distinguished from acute leukemia complicated by folate deficiency (page 574) and from sideroblastic anemia associated with folate deficiency (page 579).

### Laboratory Tests Useful in the Differential Diagnosis of Megaloblastic Anemia

#### Vitamin B<sub>12</sub> and Folate Levels in Serum and Erythrocytes

Determination of serum vitamin B<sub>12</sub> and folate levels provides a valuable means for distinguishing the two deficiency states from one another (Table 14-5). Vitamin B<sub>12</sub> levels may be determined microbiologically<sup>311,333</sup> or by isotope dilution.<sup>331</sup> Serum and erythrocyte folate concentrations usually have been determined microbiologically,<sup>328</sup> but a promising isotope dilution method has been described.<sup>310</sup> In the microbiologic assays, falsely low results may be observed in patients receiving antibiotics.

Most of the vitamin B<sub>12</sub> in serum is bound to transcobalamin I (Chapter 4) and is in equilibrium with tissue stores. Thus, the serum vitamin B<sub>12</sub> level usually is a reliable index of the total body vitamin content. There is a relatively wide difference between normal values and those found in vitamin B<sub>12</sub> deficiency (Table 14-5); however, values falling between about 120 and 180 ng/l are considered to be in a "gray zone," neither clearly normal nor clearly abnormal.<sup>23</sup> In one study, the marrow became megaloblastic when serum B<sub>12</sub> levels fell to between 70 and 154 ng/l.<sup>403</sup> The diagnostic usefulness of serum B<sub>12</sub> levels is somewhat limited by the observation that they may be subnormal in patients with folate deficiency.<sup>335,345</sup> In about half of such patients, the values were found to be less than 180 ng/l and, in about 10%, less than 100 ng/l.<sup>335</sup> In patients deficient in folate, but not in those deficient in vitamin B<sub>12</sub>, serum vitamin B<sub>12</sub> levels returned to normal when folate was administered.<sup>23,333</sup>

In contrast to serum vitamin B<sub>12</sub>, serum folate is relatively labile, being sensitive to short-term changes in folate balance.<sup>23</sup> Thus, the serum folate may increase within a few hours after consumption of folate-containing food. Furthermore, a low intake of folate may result in reduced serum levels before true deficiency develops.<sup>40</sup> The erythrocyte folate level is a better index of tissue folate stores, and its significance with respect to folate deficiency is more nearly equivalent to that

Table 14-5. Vitamin B<sub>12</sub> and Folate Levels in Normal Subjects and in Vitamin B<sub>12</sub> and Folate Deficiency

	Normal Subjects*	Vitamin B <sub>12</sub> Deficiency*	Folate Deficiency*
Serum vitamin B <sub>12</sub> <sup>23</sup> (ng/l)	450† (160-1000)†	38 (<10-110)	190 (50-500)
Serum folate <sup>344</sup> (ng/l)	10† (6-21)†	17 (4-37)	<3
RBC folate <sup>330</sup> (ng/l)	316† (166-640)†	146 (26-395)	<100

\*Values are means with range in parentheses

†Normal values may be expected to vary somewhat with the method used and from one laboratory to another

of serum vitamin  $B_{12}$  levels in vitamin  $B_{12}$  deficiency. In patients with folate deficiency, values for both serum and erythrocyte folate are found to be subnormal. In patients with vitamin  $B_{12}$  deficiency, serum folate levels tend to be increased, and, in about 25%, exceed the upper limit of normal. In contrast, erythrocyte folate levels fall (Table 14-5). This discrepancy between the two measurements presumably is the consequence of an accumulation of folate as 5-methyl tetrahydrofolate, the form which predominates in serum (see Chapter 4). Vitamin  $B_{12}$  appears to be required for the normal transfer of 5-methyl tetrahydrofolate from plasma to cells.<sup>343</sup> On the other hand, for reasons that are not clear, serum folate levels may decrease in a small proportion (2 to 10%) of vitamin  $B_{12}$  deficient subjects.<sup>23</sup>

Because of the occasional overlap in values between subjects with vitamin  $B_{12}$  deficiency and those with folate deficiency, it is usually wise to determine serum levels of both vitamins at the same time, as well as erythrocyte folate, if available. For example, in a patient with folate deficiency there may be modest reductions in serum vitamin  $B_{12}$  levels, but serum and erythrocyte folate levels are likely to be more profoundly depressed (Table 14-5). Conversely, the typical patient with vitamin  $B_{12}$  deficiency will have reduced

serum vitamin  $B_{12}$  and erythrocyte folate levels, with increased values for serum folate.

### *The Deoxyuridine (dU) Suppression Test<sup>9,334,342</sup>*

This test is a sensitive measure of a lack of 5,10 methylene tetrahydrofolate at the cellular level, an abnormality which occurs with deficiency of either folate or vitamin  $B_{12}$ . Correction of the abnormality by the addition of one of these vitamins helps to determine the nature of the deficiency. If deoxyuridine (dU) is added to normal marrow cultures, it is incorporated into DNA (Fig. 14-8). This reduces the rate of incorporation of subsequently added  $^3H$ -thymidine to less than 10% of the rate in marrow cultures without dU. With marrows collected from subjects with megaloblastic anemia, the conversion of dU to deoxythymidine monophosphate (dTMP) is inhibited (Fig. 14-8) and, therefore, a lesser degree of suppression of  $^3H$ -thymidine incorporation by dU is observed. The abnormality can be corrected by adding folic acid, whether there is either folate deficiency or vitamin  $B_{12}$  deficiency. Adding vitamin  $B_{12}$  corrects the defect only in vitamin  $B_{12}$  deficiency.<sup>9</sup> If minimally effective concentrations of the vitamins are used, selective correction only by the defi-

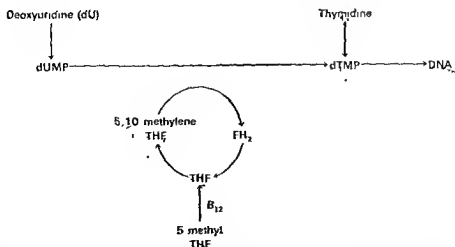


Fig. 14-8. Pathways of folate metabolism involved in the incorporation of deoxyuridine (dU) into DNA. THF, tetrahydrofolate;  $FH_2$ , dihydrofolate; dUMP, deoxyuridine monophosphate; dTMP, deoxythymidine monophosphate

cient vitamin is observed, and the procedure constitutes a "test-tube therapeutic trial."<sup>312</sup> Preliminary studies indicate that <sup>125</sup>I-uridine deoxyriboside can be substituted for <sup>3</sup>H-thymidine, making isotopic analysis considerably simpler.<sup>312</sup>

### Urinary Excretion of Metabolites

**METHYLMALONATE.** A vitamin B<sub>12</sub> coenzyme is required for the conversion of methylmalonyl coenzyme A to succinyl coenzyme A, a step in the catabolism of propionate (Chapter 4). In subjects with vitamin B<sub>12</sub> deficiency, urinary excretion of methylmalonic acid usually is increased, and this phenomenon appears to provide a specific and moderately sensitive test for the deficiency.<sup>319</sup> Unfortunately, none of the available methods is both simple and accurate enough for routine clinical use. The most accurate techniques require both ion-exchange chromatography and gas chromatography.<sup>319,323</sup> Colorimetric methods<sup>323,326,346</sup> are simpler, but less reproducible. Thin layer chromatographic methods may represent a reasonable compromise.<sup>320</sup>

Normal subjects excrete no more than 9 mg of methylmalonate in 24 hours and excretion is not increased by the prior oral administration of 10 g valine, a methylmalonate precursor.<sup>325</sup> In vitamin B<sub>12</sub> deficiency, baseline methylmalonate excretion is as much as 300 mg/day.<sup>319,325,326</sup> Occasional deficient patients may excrete normal amounts, possibly because of coincidental caloric deprivation.<sup>345</sup> In most, if not all such patients, prior administration of 10 g valine brings about a pronounced increase in urinary methylmalonate and the value then becomes abnormal.<sup>323</sup>

Normal results are obtained in patients with folate deficiency both before and after valine administration.

**FORMIMINOGLUTAMATE (FIGLU).** This compound is an intermediate in the conversion of histidine to glutamate (Chapter 4). The final step in the pathway consists of the formation of glutamate from FIGlu by the transfer of the formimino group to tetra-

hydrofolate. In folate deficiency, this step is impaired, and FIGlu is excreted in the urine. In the FIGlu excretion test, a loading dose (usually 15 g) of histidine is given and urine is collected for eight hours. Urinary FIGlu is measured most accurately by spectrophotometry.<sup>315</sup> Normal subjects excrete 1 to 17 mg of FIGlu in the eight-hour period. Excretion is almost always increased in folate-deficient subjects. In one series of 245 such patients, the value ranged from 185 to 2047 mg/8 hr.<sup>332</sup>

Increased FIGlu excretion is also found in more than 50% of patients with vitamin B<sub>12</sub> deficiency, a phenomenon that limits the diagnostic utility of the test. Usually, but not always, the amount excreted is less than that found in folate deficiency. In 16 of 18 patients with vitamin B<sub>12</sub> deficiency, FIGlu excretion ranged from 23 to 260 mg/8 hr.<sup>315</sup>

**AMINOIMIDAZOLE CARBOXAMIDE (AICAR).** This is an intermediate in purine synthesis that is metabolized in a folate-dependent step. Like FIGlu, it is excreted in excess in both folate and vitamin B<sub>12</sub> deficiency.<sup>329</sup>

### Therapeutic Trial

The uses of therapeutic trials were discussed in Chapter 13 (page 552). Although diagnosis by trial is somewhat time-consuming, the procedure is readily available where other laboratory facilities may be limited. It is essential, however, that the trial be conducted under the conditions outlined as otherwise it can be very misleading.

### Tests of Vitamin B<sub>12</sub> Absorption

Absorption of vitamin B<sub>12</sub> labeled with radioactive cobalt can be measured by several techniques.<sup>324</sup> Of the four available isotopes, <sup>57</sup>Co and <sup>58</sup>Co are the most satisfactory from the standpoint of radiation safety.<sup>339</sup> <sup>56</sup>Co and <sup>60</sup>Co have also been used, but the radiation exposure is considerably greater. <sup>57</sup>Co, a low-energy gamma emitter, is especially useful when determinations of plasma radioactivity are to be made, whereas <sup>58</sup>Co, which

emits both gamma rays and beta particles, is suitable for most other purposes, including in vivo counting over body surfaces.

In measuring absorption, a physiologic dose (usually 0.5, 1.0, or 2.0  $\mu\text{g}$ ) of the labeled vitamin is given by mouth. Absorption is estimated by assays of fecal<sup>334</sup> or urinary<sup>317,321,341</sup> excretion, serum levels,<sup>312,316,317,318</sup> total body content,<sup>314,317,327,338</sup> or hepatic uptake.<sup>322,324</sup> Without doubt, the most accurate methods are those which employ total body counting; however, the complexity and cost of the required equipment have limited the availability of the procedure. Methods depending on serum levels or hepatic uptake have the advantage of being independent of accurate collection of excreta; however, both may be affected by an abnormal distribution of the absorbed vitamin to various body sites. Fecal excretion tests require a prolonged and scrupulously complete collection of feces until all radioactivity has been excreted, which may take as long as seven to eight days. Furthermore, unless large volume counting instruments are available, the disagreeable specimens must be adequately mixed and processed so that a representative sample can be assayed.

By far, the most popular method for testing vitamin  $B_{12}$  absorption has been *Schilling's urinary excretion test*, which is comparatively simple to carry out.<sup>324,341</sup> In this test, a so-called "flushing dose" of 1000  $\mu\text{g}$  of nonradioactive vitamin  $B_{12}$  is injected intramuscularly at the same time as, or one to two hours after, 0.5 to 2.0  $\mu\text{g}$  of the labeled vitamin is given orally, and radioactivity is assayed in a urine specimen collected for 24 to 72 hours thereafter. The purpose of the injection is to partially saturate body binding sites, thereby bringing about the urinary excretion of the vitamin which would otherwise be retained. A relatively constant proportion (about 34%) of the absorbed radioactive vitamin is excreted under these conditions.<sup>23,313,317</sup> Some investigators have administered a second 1000- $\mu\text{g}$  dose of nonradioactive vitamin  $B_{12}$  24 hours after the first.<sup>321</sup> This procedure brings about the ex-

cretion of an additional small fraction of the absorbed vitamin; also, the accuracy of a subsequent test employing added intrinsic factor is improved, because the chance of contamination with label from the prior test dose is minimized.

Representative values obtained with the Schilling test are given in Table 14-6. In pernicious anemia and other disorders in which intrinsic factor is lacking, vitamin  $B_{12}$  absorption is markedly reduced, and the value is corrected toward normal by administering intrinsic factor with the orally administered vitamin. In non-tropical sprue, (gluten-induced enteropathy), the value is also reduced, although not always to the same degree as in pernicious anemia. Sprue can be distinguished from pernicious anemia by the absence of any effect of intrinsic factor. A similar pattern to that found in sprue is observed in patients with other intestinal disorders (Table 14-3, categories I-D through I-I). The values in Table 14-6 should be considered as examples only. Expected ranges will vary with the oral dose of labeled vitamin, the number of injections of "cold" vitamin, and the length of time over which urine has been collected.<sup>324</sup>

The most important source of error in the urinary excretion test is an incomplete urine collection. Another potential error is intro-

**Table 14-6. The Schilling Urinary Excretion Test of Vitamin  $B_{12}$  Absorption<sup>336</sup>**

Subjects	Urinary Excretion of Radioactive Vitamin $B_{12}$ * (%)	
	$B_{12}$ Given Alone	$B_{12}$ Given with Intrinsic Factor
Normal	18 (9-36)	—
Pernicious anemia	0.5 (0-1.2)	13 (6-31)
Non tropical sprue	3.6 (0-19)	3.3 (0-10)

\*Vitamin  $B_{12}$  dose 0.5  $\mu\text{g}$ . Urinary excretion varies with dose used as well as with other technical factors (see text)

duced by the presence of renal disease, which may delay complete excretion for as long as 72 hours. Because of these potential errors, it is useful to supplement the urinary excretion data with measurements made on plasma. Eight hours after the oral dose, 0.3 to 2.4% of the dose per liter of plasma is found in normal subjects, 0.4 to 2.24% per liter in uremic subjects, and only 0.02 to 0.15% per liter in patients with pernicious anemia. In patients with pernicious anemia given intrinsic factor the plasma level increases to 0.22 to 0.6% per liter.<sup>316</sup>

An additional disadvantage of the Schilling test is that the large parenteral dose eliminates the possibility of a subsequent therapeutic trial.

### Diagnostic Approach to the Patient

When confronted with a diagnostic problem involving macrocytic anemia, the physi-

cian should first try to distinguish between megaloblastic and non-megaloblastic anemia (Fig. 14-9). The most useful steps for this purpose are morphologic examinations. A diagnosis of megaloblastic anemia can be made on the basis of the presence of hypersegmented neutrophils and oval macrocytes in the blood or of typical megaloblasts in the marrow. These features are absent in patients with non-megaloblastic, macrocytic anemia. In the latter, the macrocytes tend to be round and, often, thin. Polychromatophilia and reticulocytosis may be prominent.

The non-megaloblastic anemias can be divided into those characterized by an increased number of reticulocytes and those in which the reticulocytes are normal or decreased in number (Fig. 14-9). These disorders are discussed in detail in Chapters 19 and 20.

In most cases of megaloblastic anemia, a preliminary diagnosis can be proposed on the basis of evidence provided by the history and

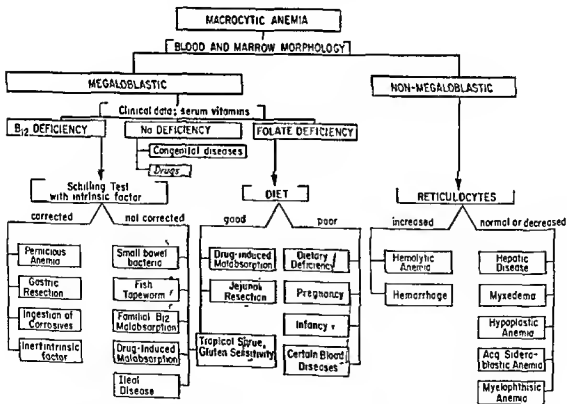


Fig 14-9 The approach to a diagnostic problem in macrocytic anemia. Closed boxes indicate findings, and open boxes represent procedures. (For discussion, see text.)

the physical examination. Particular attention should be paid to the patient's age, adequacy of diet, association with pregnancy, history of gastrointestinal operations, symptoms of steatorrhea, exposure to medications, alcoholism, family history, and the presence of neurologic disease.

Two common examples may be used to illustrate the usefulness of this kind of information. Megaloblastic anemia appearing in a previously healthy, well-nourished, elderly individual is most likely to result from pernicious anemia. On the other hand, the same type of anemia in a poorly nourished woman in her last trimester of pregnancy is almost certainly the consequence of folate deficiency.

For the difficult case, the sophisticated laboratory methods discussed above may be employed for precise diagnosis. The proper selection and interpretation of these tests depend upon the physician's careful analysis of the clinical data. In the above example of probable pernicious anemia, there is no need to obtain serum vitamin levels or to measure methylmalonate excretion. The diagnosis can be confirmed by measurement of vitamin B<sub>12</sub> absorption with and without added intrinsic factor.

Only in those patients with obscure or complex illnesses will a battery of biochemical procedures be required. In such circumstances, it is best to try to determine whether there is a deficiency of vitamin B<sub>12</sub>, of folate, or of neither (Fig. 14-9). There are no reliable morphologic criteria for this purpose. The detection of *subacute combined degeneration* of the spinal cord (see Chapter 15, page 606) is of importance because, with one possible exception,<sup>337</sup> this condition occurs only in vitamin B<sub>12</sub> deficiency; however, the absence of this sign is not helpful, since it now is an uncommon finding even in classical pernicious anemia.

Selection of appropriate laboratory methods to document vitamin deficiency depends to some extent on availability. Serum vitamin levels represent the most direct and reliable determinations for this purpose. If they are unavailable, it may be necessary to resort to therapeutic trial, as discussed in Chapter 13.

Vitamin B<sub>12</sub> deficiency states can be divided into those that are and those that are not due to absence of intrinsic factor, as indicated by the Schilling test (Fig. 14-9). Diagnosis of a folate deficiency state usually depends primarily on a carefully elicited history that includes information permitting critical evaluation of dietary intake (Fig. 14-9).

## Management

In most cases of megaloblastic anemia, the most important goal of therapy is the repletion of body stores of the deficient vitamin. Care should be taken to define the nature of the deficiency with precision so that the proper vitamin can be given in adequate amounts and over a sufficiently long period of time. To administer the wrong vitamin or a mixture of agents is not only wasteful and expensive, but also potentially dangerous. For example, therapeutic doses of folic acid may induce transient hematologic improvement in patients with vitamin B<sub>12</sub> deficiency, but the response is suboptimal, and, furthermore, the neurologic manifestations of vitamin B<sub>12</sub> deficiency may appear and progress during treatment.<sup>428</sup> Conversely, the folate-deficient patient may respond suboptimally to vitamin B<sub>12</sub>.<sup>347,460</sup>

In addition to therapy with vitamin B<sub>12</sub> or folate, it is sometimes possible to treat the underlying disease, eg, by surgical correction of anatomic lesions leading to small bowel bacterial overgrowth,<sup>311</sup> expulsion of the fish tapeworm,<sup>334</sup> discontinuation of drugs leading to vitamin malabsorption,<sup>118</sup> the use of a gluten-free diet in non-tropical sprue,<sup>275</sup> or by administration of antibiotics in tropical sprue.<sup>277</sup>

General measures appropriate to the treatment of all anemias have been discussed in Chapter 13 (page 550).

## Blood Transfusions

Transfusions are rarely required in megaloblastic anemia. Even when the anemia is severe, the likelihood of obtaining a dramatic response to specific therapy within 48 to 72



hours generally makes it unnecessary to subject the patient to the risks, discomfort, and expense of blood transfusion (Chapter 11). Sometimes, however, transfusion may be desirable and even life-saving. The most important indications for transfusion are signs of circulatory collapse, high-output cardiac failure with pulmonary congestion at rest, or severe or intractable angina. Mental deterioration is another indication, but a less definite one since this can be a manifestation of vitamin deficiency rather than cerebral anoxia. These situations must be treated as acute emergencies. In one series of 108 patients with megaloblastic anemia, the mortality rate was 14%, mostly because severe cardiac failure or myocardial infarction occurred before a therapeutic response was attained.<sup>47</sup>

While blood is being given, appropriate indicators of cardiac and circulatory function should be monitored frequently, including central venous or pulmonary wedge pressure. Fresh blood is to be preferred, to ensure optimal oxygen delivery capability (page 473). Only the packed red cells should be given. The rate of administration must be no greater than about 250 ml per four to six hours, and the patient should be kept in a sitting position. In unusually serious circumstances, "exchange transfusion" has been used, blood of low red cell content being removed from one arm while packed red cells are administered in the other.<sup>417-420</sup>

### *Therapy with Vitamin B<sub>12</sub>*

In 1926, Minot and Murphy demonstrated the value of liver therapy in pernicious anemia.<sup>433</sup> The induction of remission required the daily consumption of a half pound of liver, a regimen that severely tested the endurance of these patients! Desiccated whole hogs' stomach, mammalian kidney and brain,<sup>411,416</sup> and brewer's yeast,<sup>458</sup> all of which were shown to be effective remedies in this disease, are now only of historic interest. Even the highly effective, concentrated liver extracts that were developed in the 1930's now find no use.<sup>415</sup> Completely satisfactory results can be achieved by adminis-

tration of vitamin B<sub>12</sub>, which is both cheaper and less troublesome to administer than the older preparations.

**PREPARATIONS AND SIDE EFFECTS.** Two forms of vitamin B<sub>12</sub> are available for therapeutic use, namely, cyanocobalamin and hydroxocobalamin, the chemical structures of which are given in Chapter 4. Both are capable of inducing and maintaining remissions in vitamin B<sub>12</sub> deficiency states, and both are non-toxic except for very rare allergic reactions.<sup>430</sup> At high doses they differ in the degree to which they are retained in the body and excreted in the urine, as will be discussed below. Both forms of vitamin B<sub>12</sub> are available as solutions containing either 100 or 1000 µg/ml for intramuscular or subcutaneous use. Depot preparations, such as cyanocobalamin zinc tannate or cyanocobalamin-tannate in sesame oil-aluminum monosterate gel, have been marketed, but these offer no particular advantages over hydroxocobalamin.

**STORAGE AND EXCRETION.** The normal body stores of vitamin B<sub>12</sub> have been estimated to be between 3.5 and 11 mg (average 5 mg) (Chapter 4).<sup>414,415</sup>

In patients with signs of deficiency, stores have been estimated at 0.5 mg (0.1 to 0.7).<sup>403</sup> It is not possible to restore the normal body reserves by a single injection of vitamin B<sub>12</sub>, even if massive amounts are given, because only a portion of an injected dose is retained, and that amount is inversely related to the size of the dose. Of quantities up to 50 µg of cyanocobalamin, about 90 to 95% will be retained 48 hours after injection, but only about 45% of a 100-µg dose and only 15% of 1000-µg dose will be retained in the same period.<sup>402,412,414,431,435,438,443</sup> Most of the remainder is lost in the urine, principally during the first 24 hours after the injection.<sup>408</sup> Urinary losses continue at a moderate rate for another four to eight weeks before reaching a low, long-term rate of loss.<sup>407</sup> When hepatic disease is present the daily losses of cobalamin from body stores are about doubled. Even when as much as 5 mg of cyanocobalamin is given intravenously as a single

dose, only about 450  $\mu\text{g}$  remain in the body 10 days later.<sup>407</sup> If one or more mg of vitamin  $\text{B}_{12}$  are to be stored following parenteral administration, a number of injections separated from one another by at least 24 hours are required, rather than a single dose. Although large doses are not harmful, smaller doses allow more efficient conservation of the injected vitamin.

The amount of a larger dose that is retained depends upon the form of vitamin  $\text{B}_{12}$  that is injected. The initial retention of hydroxocobalamin is better than that of cyanocobalamin.<sup>410,422,442,449</sup> In the first 24 to 72 hours after injection, two to three times more hydroxocobalamin than cyanocobalamin is retained.<sup>402,408</sup> Twenty-eight days after the injection, although the amount of hydroxocobalamin retained has decreased by one fourth to one half, retention still is nearly three times greater than the retention of cyanocobalamin. After the fourth to eighth weeks, both cobalamins appear to be lost from the body at about the same rate (about 0.1 to 0.2% of body stores per day),<sup>401,407,450</sup> possibly because cyanocobalamin becomes converted to hydroxocobalamin *in vivo*.<sup>439</sup>

Despite the fact that, in the average patient, hydroxocobalamin provides higher serum concentrations of vitamin  $\text{B}_{12}$  for a longer period than does cyanocobalamin,<sup>410</sup> there is considerable individual variation in the duration of response. "Short responders" have been identified who do not maintain their serum  $\text{B}_{12}$  concentrations very well, no matter what compound is injected.<sup>449</sup> In these patients the serum  $\text{B}_{12}$  concentration may fall to 140 ng/l two weeks after hydroxocobalamin has been administered, about one eighth the time it takes for this to occur in the usual patient. Hematologic and neurologic relapses may occur when serum concentrations of vitamin  $\text{B}_{12}$  reach the neighborhood of 70 to 150 ng/l.<sup>403</sup>

**ROUTES OF ADMINISTRATION AND DOSAGE SCHEDULES.** Parenteral injections of as little as 1  $\mu\text{g}$ /day of vitamin  $\text{B}_{12}$  will induce a complete hematologic remission in pernicious anemia.<sup>447</sup> Doses of this size are recom-

mended when therapeutic trials are performed for diagnostic purposes (Chapter 13) because they will have no effect in folate-deficiency states. However, if vitamin  $\text{B}_{12}$  stores are to be replenished, considerably larger doses are required.

Recommendations regarding the compound, dose, and frequency with which vitamin  $\text{B}_{12}$  is injected for induction of remission, restoration of body stores, and maintenance of clinical remission vary. Although there is much latitude in therapy, it is necessary that the patient and his blood be examined frequently during the initial phase, in order to determine that remission is being achieved. Thereafter the patient should be examined every three to six months to make certain that remission is being maintained.

The following schedule has been used successfully for years as the initial treatment for pernicious anemia in relapse and for the reestablishment of body stores of vitamin  $\text{B}_{12}$ . Similar regimens may be employed for other vitamin  $\text{B}_{12}$  deficiency states. At first, 100  $\mu\text{g}$  of cyanocobalamin are given intramuscularly, daily, for six or seven days. Reticulocyte counts and simple clinical observations are made at the time of each injection. The characteristic clinical and reticulocyte response described below should occur within the week. If that is the case, the same amount is given on alternate days for another seven doses, and the hematocrit is measured twice weekly during that time. A distinct increase should be evident. After this, the injections are continued every third or fourth day for another two to three weeks, thereby providing a total of 1.8 to 2.0 mg  $\text{B}_{12}$  in five or six weeks. Such a schedule ensures that much of the administered  $\text{B}_{12}$  has been retained. By this time, hematologic values should have become normal.

Alternatively, only five weekly injections of 1 mg each of hydroxocobalamin are given,<sup>422</sup> depending on the desirability in the individual case for the patient to return for medical supervision. Early discovery of a patient's failure to respond promptly and completely allows a mistaken diagnosis to be recognized or interfering factors to be identi-

fied,<sup>447</sup> and corrective action may then be taken before further complications ensue.

Maintenance therapy with cyanocobalamin consists of monthly injections of 100  $\mu\text{g}$  for life. It is possible that the use of hydroxocobalamin may permit extension of the interval between injections,<sup>422,442,444</sup> although such a regimen might be a disservice to the "short responders" described in the previous section.<sup>449</sup> With hydroxocobalamin, 1.0 mg may be injected every two to four months and examinations made at least every four months to ascertain that the remission is being maintained. Alternatively, vitamin B<sub>12</sub> levels may be measured periodically to identify those patients who require frequent therapy.

Under exceptional circumstances, vitamin B<sub>12</sub> is given orally.<sup>431,433</sup> Candidates for oral therapy include patients who refuse parenteral injections or those in whom parenteral therapy may be hazardous, eg, when there is a coexisting disorder of hemostasis. Also, rare patients have been reported who exhibited hypersensitivity reactions to the vitamin when it was given parenterally, but not orally.<sup>421</sup> In pernicious anemia subjects, only about 1% of orally administered vitamin B<sub>12</sub> is absorbed, presumably by routes not dependent on intrinsic factor. Consequently, doses that are very large in relation to the body's requirements must be given, usually 300 to 1000  $\mu\text{g}/\text{day}$ . Another, considerably less satisfactory approach to oral therapy is the use of preparations containing vitamin B<sub>12</sub> and porcine intrinsic factor. Although this combination facilitates vitamin B<sub>12</sub> absorption at the outset, patients eventually become refractory to the action of such heterologous intrinsic factor because of the development of antibodies.<sup>405</sup> For this reason, and also because of greatly increased cost, these preparations have no legitimate therapeutic uses.

Patients receiving vitamin B<sub>12</sub> orally should receive closer supervision than those who receive the vitamin parenterally.<sup>431</sup> In all patients, the risk of hematologic and neurologic relapse, because of forgotten or otherwise omitted treatment, is ever present.

## Therapy with Folates

The first use of preparations containing folates was in the early 1930's when Wills and her colleagues found that the megaloblastic anemia of pregnant, Indian women responded to autolyzed yeast (marmite), or to a component of crude liver extracts that could be distinguished from the anti-pernicious anemia principle.<sup>456</sup> When crystalline folic acid became available in 1945 it almost entirely replaced the crude substances that were formerly used.

**PREPARATIONS.** The form of folate most commonly used for therapy is pteroylmonoglutamic acid, also known as folic acid. Prior to 1971, the standard oral folic acid tablet contained 5 mg, an amount that is not only excessive, but also wasteful since much of it is excreted. Presently available in the United States are tablets of 1.0, 0.15, and 0.1 mg. Folic acid is also available in a solution of 5 mg/ml for parenteral use. *Folinic acid* (N<sup>5</sup>-formyltetrahydrofolic acid) is available in solutions intended for parenteral use. Recent studies indicate that it may also be effective when given by mouth.<sup>436</sup>

**ABSORPTION, STORAGE, AND EXCRETION.** The normal body folate stores have been estimated at 5 to 10 mg, an amount sufficient for only about two to four months of normal hematopoiesis.<sup>40</sup> Stores of this magnitude can be maintained in normal subjects by a dietary intake of 50 to 100  $\mu\text{g}/\text{day}$ . The folates normally found in foodstuffs are polyglutamates and must be converted to the monoglutamate prior to absorption (Chapter 4). This conversion may be defective in many of the folate deficiency states, especially those associated with certain drugs and with malabsorptive disorders. On the other hand, the absorption of pteroylmonoglutamic acid in patients with these disorders may be normal or nearly so.

In normal subjects, approximately 80% (range 40 to 95%) of orally administered folic acid is absorbed, regardless of dose.<sup>404</sup> When folic acid is administered in doses less than 200  $\mu\text{g}$  per day, little or none is lost in the

urine. At higher doses, urinary losses are considerable. About 6% of a 1-mg dose, 10% of 2-mg, 50% of 5-mg, and 80% of 15-mg are excreted.<sup>23,413</sup>

**DOSES AND ROUTES.** An optimal hematologic response will occur if 50 to 100  $\mu$ g of folic acid are given daily by mouth. Such doses are useful for therapeutic trials (Chapter 13). However, for replenishment of stores, larger amounts are required. A daily dose of 1 mg for two to three weeks should be ample, even in patients with malabsorption. It is most unlikely that a patient who fails to respond to oral administration will respond to injections. The parenteral forms are useful chiefly in patients who are unable to take medications by mouth.

Once stores have been repleted, the need for maintenance therapy must be considered in the light of the underlying condition that produced the folate deficiency. If that condition can be corrected, normal dietary sources of folate should suffice. When the causative condition cannot be reversed, eg, when requirements are increased owing to a persisting severe hemolytic anemia or in pregnancy, when malabsorption cannot be corrected, as by a gluten-free diet, or when dietary habits are inadequate and immutable, appropriate supplements in the range of 0.1 to 0.5 mg daily must be given. The usual "prophylactic" dose in pregnancy is 0.3 mg/day. When the continued administration of diphenylhydantoin is necessary, 0.6 to 1 mg of oral folic acid daily may be required. Life-long therapy with folate is rarely necessary, but, if it is contemplated, vitamin B<sub>12</sub> absorption must be known to be normal (see below).

*Folinic acid* is the preparation of choice for the treatment of toxicity caused by folate antagonists. These agents inhibit the reduction of folic acid by dihydrofolate reductase. Folinic acid, being already fully reduced, bypasses this metabolic block. The usual dose is 3 to 6 mg given intramuscularly. Folinic acid also has been employed successfully in patients with congenital dihydrofolate reductase deficiency.<sup>304</sup>

**UNTOWARD EFFECTS.** Folic acid is remarkably free of toxic effects, except for very rare instances of allergic reactions.<sup>459</sup> In some epileptics, the frequency of seizures appears to have been aggravated by folate.<sup>411</sup>

By far the most important contraindication to folic acid therapy is the presence of an untreated deficiency of vitamin B<sub>12</sub>. As previously noted, a suboptimal hematologic response to folate may occur in such situations, but neurologic disease is not relieved, and, in fact, may appear and progress.<sup>428</sup> Of equal concern is the possibility that vitamin B<sub>12</sub> deficiency will develop at a later time during a long course of folate therapy. In this situation, hematologic manifestations may not develop and only neurologic disease will appear. It is therefore prudent, whenever possible, to perform tests of vitamin B<sub>12</sub> absorption whenever long-term folate therapy is contemplated. If absorption is defective, prophylactic vitamin B<sub>12</sub> therapy is justified.

#### *Response to Treatment with Vitamin B<sub>12</sub> or Folate*

In general, those aspects of disease that can be ascribed to vitamin deficiency are rapidly corrected by vitamin B<sub>12</sub> or folate therapy, but signs and symptoms of the underlying disease are not relieved. For example, in pernicious anemia, there is no regeneration of gastric cells; achlorhydria and other aspects of achylia gastrica are unchanged; and vitamin B<sub>12</sub> absorption remains profoundly impaired.

**SYMPTOMS.** Symptomatic improvement often may be recognized before any change in the blood is noticeable. The patient becomes more alert, more cooperative, and his appetite improves. These signs may be observed within 48 hours of adequate parenteral therapy. If soreness of the tongue is present, it also is usually relieved within 48 hours; regeneration of the papillae is observed within four to seven days, and the tongue becomes normal after two to three weeks.<sup>440</sup>

**BLOOD.** An increase in reticulocytes is the earliest and most useful sign of hematologic

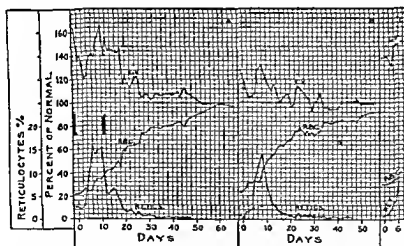
**Table 14-7. The Relation of Reticulocyte Count at Its Peak to the Degree of Anemia in Patients with Pernicious Anemia Treated with Vitamin B<sub>12</sub><sup>23</sup>**

Initial Red Cell Count (cells $\times 10^{12}/l$ )	Reticulocytes at Peak (%)
<1.0	50-70+
1.0-1.49	36-47
1.5-1.99	25-34
2.0-2.49	15-22
2.5-2.9	10-16
3.0-3.6	4-9

response to therapy. With optimal therapy, the increase becomes apparent after two or three days and maximum numbers are reached on day five to eight. The magnitude of the reticulocytosis is related to the degree of anemia (Table 14-7). After the peak reticulocyte response has been reached, there is a gradual fall that may sometimes be interrupted by a second rise (Fig. 14-10). Nucleated red corpuscles may appear in the blood when the reticulocyte response occurs.

After a delay of about five to seven days, a perceptible increase in the VPRC is observed. The rate of increase is greatest in those patients with the most severe anemia (Fig. 14-11). If no complications develop, normal values should be attained by four to eight weeks, regardless of the initial degree of anemia.<sup>427</sup> Mean corpuscular volume, after a preliminary increase due to the increased numbers of reticulocytes,<sup>457</sup> gradually decreases (Fig. 14-10) and values return to normal at about the same rate as the red cell count becomes normal. Simultaneously, the marked anisocytosis decreases and the numerous bizarrely shaped erythrocytes disappear.

The neutrophilic leukocytes increase in number, usually reaching normal values within a week. Immature white cells may accompany the increase; myelocytes and even a rare myeloblast may be found. In rare instances, the temporary "shift to the left" may be so pronounced that the blood picture of myelocytic leukemia is simulated.<sup>418</sup> Hypersegmented leukocytes gradually disappear and usually are absent by 14 days.<sup>23</sup> The



**Fig. 14-10.** Variations in mean volume of red corpuscles compared with reticulocyte count in three patients with pernicious anemia. The mean corpuscular volume (CV) and the red cell count (RBC) are represented as percent of their respective average normal values. By this method the red cell count and mean corpuscular volume of a hypothetical normal individual would fall on the line at 100%. Reticulocytes are recorded directly. The abscissa records days following the commencement of liver therapy (From Wintrobe,<sup>26</sup> courtesy of Journal of Clinical Investigation.)

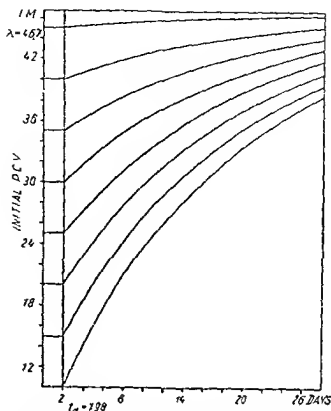


Fig. 14-11 Expected course of the VPRC (PCV) after the intramuscular administration of vitamin  $B_{12}$  to patients with pernicious anemia. There always is a lag averaging 1.98 days ( $t_0$ ) before a perceptible increase occurs. Ultimately an average VPRC value of 0.467 l/l ( $\lambda$  46.7) is reached. (From Waelsch and Sidak,<sup>452</sup> courtesy of the authors and *Folia Haematologica*.)

platelet count returns to normal within a week.<sup>437</sup>

If therapy is suboptimal or if complicating disease, eg, infection, is present, the typical response will be impaired.<sup>23</sup> In particular, the magnitude of the reticulocytosis will be reduced and the time at which peak values are reached will be delayed. The increase in VPRC also will be less rapid.

**BONE MARROW.** Following effective therapy, the morphologic appearance of the bone marrow is altered with extraordinary rapidity. Within six to ten hours, the number of megaloblasts may become greatly decreased.<sup>416</sup> By 24 to 48 hours, erythrocyte maturation is normoblastic.<sup>445</sup> The effect of therapy on individual megaloblasts has been disputed. It has been proposed that these abnormal cells can be converted directly to normal ones by supplying the missing vita-

min. On the other hand, sequential kinetic studies have suggested that the marrow is repopulated by an entirely new series of normoblasts from the stem cell level, and that the remaining megaloblasts are destroyed within the marrow.<sup>426</sup> Giant metamyelocytes may not disappear from the marrow for a week or more.<sup>23</sup>

**BIOCHEMICAL CHANGES.** The increased plasma iron concentration characteristic of megaloblastic anemia in relapse decreases over a 24- to 48-hour period. Distinctly subnormal levels may be reached.<sup>424</sup> The serum iron may remain low for several weeks. In patients with treated vitamin  $B_{12}$  deficiency, a similar pattern has been observed in serum folate levels; during the first 24 hours of therapy, a decrease from an average of 15.4 to 5.5 mg/l was observed, followed by a gradual increase over the next two weeks.<sup>454</sup>

Increased urinary methylmalonate levels (page 586) decrease in 48 hours and return to normal within a week.

The plasma bilirubin falls at about the end of the first week and usually will be found within normal limits at the end of three or four weeks. Urinary urobilinogen begins to decrease at the end of the reticulocyte response.<sup>419,425</sup> Free erythrocyte protoporphyrin tends to increase with therapy.<sup>435</sup> Megaloblastic bone marrow was found to contain no protoporphyrin,<sup>443</sup> but, with the re-appearance of normoblasts following therapy, protoporphyrin was detected in marrow and its quantity increased until the maximum reticulocyte value was reached; thereafter, the marrow protoporphyrin decreased slightly.

The serum urate level increases as reticulocytosis occurs, presumably because of accelerated turnover of DNA in rapidly proliferating erythrocyte precursors.<sup>409</sup> The increase may be prevented by the administration of the xanthine oxidase inhibitor, allopurinol. Urinary phosphorus decreases rapidly when the appropriate vitamin is first given, increases during reticulocytosis, and then gradually returns to normal values.<sup>429</sup> These changes probably are related to increased phosphorus uptake in association with the formation of increased quantities of cellular nucleotides and polynucleotides.

Serum potassium concentration falls an average of 0.9 mEq/l during the first 48 hours of therapy and then gradually returns to normal over the next several weeks.<sup>47</sup>

Serum LDH levels return to normal within one to two weeks.

**NEUROLOGIC MANIFESTATIONS.** Although progression of the neurologic disease in pernicious anemia is halted by vitamin B<sub>12</sub> therapy, the degree to which established lesions can be reversed varies. In general, the shorter the duration of the sign or symptom, the more likely it is to disappear with treatment; neurologic manifestations that have been present less than three months are usually reversible.<sup>405</sup> Symptoms of long duration may be lessened by treatment, but some residual dysfunction is to be expected. Neurologic improvement tends to be relatively

slow. Often six months or more elapse before a maximum response is achieved. As a rule of thumb, manifestations persisting after a year of optimal therapy may be considered irreversible. In a series of 44 patients with subacute combined degeneration, most of the observed neurologic abnormalities returned to normal or improved in 80 to 90% of patients.<sup>451</sup>

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## Pernicious Anemia

History  
Incidence  
Etiology and Pathogenesis  
Clinical Manifestations  
Laboratory Findings  
Course, Prognosis, and Complications  
Pathology

**P**ERNICIOUS anemia is a chronic illness resulting from the lack of Castle's "intrinsic factor" in gastric secretions. In the absence of this gastric binding protein, absorption of vitamin B<sub>12</sub> is markedly impaired, and deficiency of the vitamin ultimately supervenes. The disease occurs in two forms: a relatively common adult type and a rare congenital variety. In the adult type, the lack of intrinsic factor is associated with gastric atrophy and deficiency of many other gastric secretions (achylia gastrica). In the congenital form, only intrinsic factor is lacking, other components of gastric juice remaining normal.

### History

The first (1855) clinical description of pernicious anemia is usually attributed to Thomas Addison,<sup>1</sup> but probable cases were recorded in 1823 by Combe and Andral, and in 1837 by Marshall Hall.<sup>2</sup> In his somewhat vague description, Addison used the term "idiopathic anemia," indicating that the diagnosis was made by excluding other, known

causes of anemia.<sup>1</sup> The term "pernicioser" was used by Biermer,<sup>2</sup> but, ironically, probably only one of his 15 patients had vitamin B<sub>12</sub> deficiency.<sup>3,12</sup> The anglicized version of Biermer's designation became popular after the term appeared in an 1876 editorial in an English medical journal.<sup>3,8</sup>

Even as early as 1860, Austin Flint expressed the view that "Addisonian anemia" results from deficient gastric secretions and consequent inadequate assimilation of food.<sup>9</sup> In 1870, Fenwick reported an autopsy study of a person whose gastric glands were found to be atrophic; the acidified mucosal scrapings failed to digest egg white.<sup>10</sup> Although achylia gastrica then came to be recognized as a consistent finding, and one which antedates the development of anemia, the pathogenetic significance of the observation remained a mystery.

Fifty years later, Whipple, while seeking to determine the antianemic effectiveness of various foods (Chapter 13), demonstrated the value of liver.<sup>11</sup> His observations led Minor and Murphy to embark on a therapeutic trial of giving large amounts of liver by mouth to patients with pernicious anemia. They reported in 1926 that the anemia could be promptly relieved by this regimen.<sup>13</sup> Before this, patients with pernicious anemia had been treated with an "iron-rich" diet—based on Whipple's observation—that contained liver,<sup>11</sup> and "liver soup" had been used in the treatment of tropical sprue. However, the

unique value of liver had not been appreciated before Minot and Murphy conducted their systematic study. The daily measurement of reticulocytes before therapy and the demonstration of a striking reticulocyte response associated with the feeding of liver made Minot and Murphy's findings convincing.

The investigations of Whipple and his associates, and those of Minot and of his pupil, William Castle, transformed hematology from a field concerned solely with morphology to the dynamic discipline it is today.<sup>4</sup> As will be discussed later (page 604), Castle's experiments clarified the relationship between achylia gastrica and the effectiveness of liver.<sup>3,6</sup>

## Incidence

### (i) Race

The adult form of pernicious anemia is particularly common among individuals of Scandinavian, English, and Irish ancestry<sup>26</sup> (Fig. 15-1). In such high-risk groups, about nine new cases are detected per 100,000 population per year, and about 0.13% of the population is affected.<sup>33</sup> The disease appears to be much less common in Caucasians of Italian or Greek origin.<sup>26</sup> Although once thought to be rare among American Indians, pernicious anemia was found to be as common among members of 10 Southwestern tribes as it is in Caucasians.<sup>34</sup> In studies of

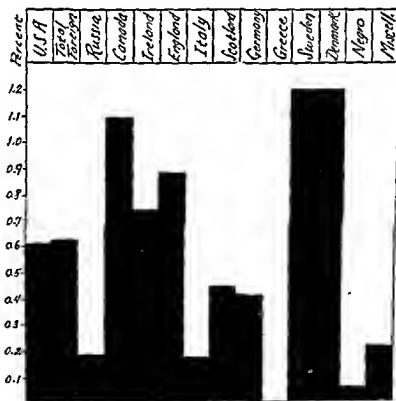


Fig 15-1. Prevalence of pernicious anemia in natives of various countries, as indicated by 500 admissions from 1913 to 1932 to the Peter Bent Brigham Hospital, Boston. The values in percent indicate the proportion of cases of pernicious anemia among the total admissions from the various countries (From Friedlander,<sup>26</sup> courtesy of the author and American Journal of Medical Sciences.)

hospitalized patients, its prevalence in American Negroes was found to be about one third (Fig. 15-2) to one half of that in Caucasians.<sup>27</sup> It has been reported, albeit rarely, in the South African Bantu,<sup>31</sup> in Orientals,<sup>22,25,29</sup> and in Arabs.<sup>21</sup>

### Sex

A clear female preponderance has been observed in Great Britain and Scandinavia (1.7 to 2.0:1 or greater).<sup>23</sup> More nearly equal distribution between the sexes has been observed in the United States, and, in one large series, there was a slight male preponderance (Fig. 15-2).

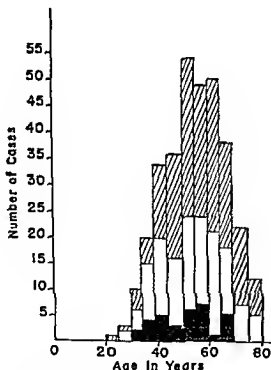


Fig 15-2 Age, sex, and race of 329 patients with pernicious anemia admitted to the Johns Hopkins Hospital from 1925 to 1940. Negro patients of both sexes are represented together in the black portion of each column, white men are indicated by the hatched portion of each column, white women by the open portion. The height of each column represents the total number of patients of each age group. There were 171 white men, 125 white women, and a total of 33 Negroes, of which 20 were men and 13 women. The ratio of all white to Negro admissions at this hospital was about 3 to 1.

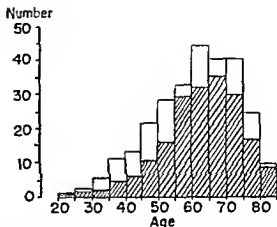


Fig 15-3. Incidence of pernicious anemia at various ages calculated according to number of cases per 100 000 population. Females hatched columns; males open columns. (From data for Sweden collected by Nordenson et al.<sup>32</sup>)

### Age

Pernicious anemia is most common in persons in late adult life. It is rare in individuals younger than 30 years of age and increases in frequency as age advances (Fig. 15-3). The average age of onset in one large series was 60.5 years.<sup>207</sup> In 1944, only four persons less than 20 years old were found among a total of 1532 pernicious anemia patients.<sup>24</sup> Since then, more cases in children have been reported. These appear to fall into two groups.<sup>30,25</sup> The illness in one group (about 27 patients) is designated "congenital" pernicious anemia, because it is usually apparent before the subject is two years of age. It is characterized by a lack of intrinsic factor, but the structure and function of the gastric mucosa are otherwise normal. In the second, even smaller group, having so-called "juvenile" pernicious anemia, onset has occurred in the second decade of life. Gastric atrophy is found and the illness seems to be the same in all respects as the adult disease.

### Etiology and Pathogenesis

Castle and his associates showed that a hematologic response in pernicious anemia could be achieved by administering beef

muscle previously incubated with normal human gastric juice, but that neither the beef nor the gastric juice was effective alone.<sup>6</sup> As a result of these experiments, Castle proposed that beef contains an essential "extrinsic factor" which combines with gastric "intrinsic factor" to form an antianemic principle that is stored in the liver. When vitamin B<sub>12</sub> was isolated, it was shown that this vitamin is both the extrinsic factor and the antianemic principle, and that gastric "intrinsic factor" is a binding protein essential to vitamin B<sub>12</sub> absorption. The metabolism of vitamin B<sub>12</sub> and the action of "intrinsic factor" have been discussed in Chapter 4.

In adult pernicious anemia, the fundamental defect is severe gastric atrophy with loss or extreme deficiency of all gastric secretions, including Castle's intrinsic factor. The cause of the gastric lesion remains unknown and continues to be the subject of active investigation. It is likely that inherited factors play a role in the illness, but the relatively late age at which the onset occurs and the lack of a clear hereditary pattern suggest that acquired, environmental influences must interact with the genetic defect.<sup>145</sup> Among the acquired factors is simple gastritis, a usually asymptomatic disease that appears to be extremely common and tends to progress with age. Also, immunologic mechanisms have been implicated in pernicious anemia by its association with certain "autoimmune" disorders and by the presence of antibodies directed against gastric antigens. Each of these topics will be discussed below.

### Genetic Factors

It seems clear that the congenital form of pernicious anemia is inherited, probably as an autosomal recessive trait.<sup>30,35</sup> Observations supporting these conclusions are: (1) the early age of onset, (2) a high incidence of consanguinity among the parents, and (3) the frequency with which siblings have been affected (10 of 27 families). There is no good evidence, however, of a genetic relation between the congenital and adult forms of pernicious anemia. With one exception, no in-

stances of adult pernicious anemia have been reported in families of patients with congenital pernicious anemia.<sup>35</sup> Furthermore, congenital pernicious anemia has not occurred in any members of the 15 reported families in which both parents had the adult form of the disease.<sup>23</sup>

Compilation of various studies of the familial incidence of adult pernicious anemia indicates that, within the families of 2246 patients, multiple occurrences of pernicious anemia could be demonstrated in 286 instances (12.7%).<sup>23</sup> In these studies, the proportion of families with more than one case of pernicious anemia ranged from 7.9%<sup>139</sup> to 30%.<sup>129</sup> It is possible that the higher figures are the more accurate ones, since they represented more thorough studies and included persons in older age groups.

It follows that the risk of developing the disease in relatives of patients with pernicious anemia is considerably greater than that of the general population. The overall prevalence of pernicious anemia among children, siblings, parents, and parent's siblings of patients with pernicious anemia is about 2.5%, or about 20 times the prevalence in the population at large.<sup>129</sup> The figure becomes even greater if age and closeness of relationship are considered. Of members of the highest-risk group (siblings), about 21% may be expected to acquire pernicious anemia before their ninetieth birthday.<sup>129</sup>

Eighteen instances of the concordant occurrence of pernicious anemia in twins have been reported<sup>105,114</sup>; however, in three other sets of twins the disease occurred in only one of each pair. These data appear to support a genetic origin for pernicious anemia. However, they must be interpreted with caution, for in many of the reports the evidence for the diagnosis or for the identity of the twins was inadequate.<sup>105</sup> Furthermore, even a single instance of failure of an identical twin to acquire his sibling's disease emphasizes the importance of nongenetic factors.

Also cited in support of a genetic origin for pernicious anemia is an increased incidence of some features of the disease in relatives. For example, in one study,<sup>109</sup> achlor-



Hydria was found in 27% of the relatives of patients with pernicious anemia. Although this incidence was not clearly different from that in age-matched controls, 8% (10 of 124) of the achlorhydric relatives were found to have both impaired vitamin B<sub>12</sub> absorption and reduced serum levels of the vitamin. In another investigation, partial defects in vitamin B<sub>12</sub> absorption, as measured by the Schilling test, were reported in relatives of pernicious anemia subjects.<sup>128</sup> However, according to a later study, inadequate urine collection may have been responsible for this finding.<sup>127</sup>

More convincing is the evidence that relatives of pernicious anemia patients are predisposed toward the production of autoantibodies to gastric parietal cells (see below). If the results of several series are combined, of 763 relatives examined, 24% (30% of the women and 18% of the men) were found to have circulating parietal cell antibodies.<sup>23</sup> The sera of only 3.8% of subjects in the control series gave positive evidence of the presence of these antibodies. In relatives of pernicious anemia patients, there may also be an increased incidence of certain diseases that occur with increased frequency in patients with pernicious anemia (page 608).<sup>145</sup>

Despite these studies, the pattern of inheritance remains in doubt. Both dominant and recessive modes have been suggested, but the incomplete penetrance of the trait has precluded rigorous proof of either. The nature of the genetic lesion remains unknown, but two major possibilities have been suggested<sup>113</sup>: (1) that it is a defect in immunologic tolerance to a particular group of antigens found in the stomach, thyroid gland, pancreas, and skin; and (2) that it is a defect in a metabolic or enzyme system common to these tissues.

It has been suggested that adult pernicious anemia may exist in two forms, inherited and acquired.<sup>120</sup> According to this hypothesis, in contrast to the acquired form the inherited form is characterized by (1) an earlier age of onset (average 51 vs. 66 years), (2) a characteristic association with certain other diseases (page 608), and (3) somewhat lower serum immunoglobulin levels.

## Gastritis

A high proportion of otherwise normal individuals over the age of 30 have been found to have histologic evidence of gastritis on gastric biopsy.<sup>121</sup> Both the incidence and the severity of gastritis increase with age (Fig. 15-4). At least three histologic stages of progression have been defined: (1) superficial gastritis, (2) atrophic gastritis, and (3) gastric atrophy. In the last two stages, glandular structures are destroyed, and there is a progressive loss of gastric secretions. Characteristically, hydrochloric acid, pepsin, and intrinsic factor are lost in that order.<sup>15</sup> Patients with severe degrees of atrophic gastritis or with gastric atrophy may develop impaired vitamin B<sub>12</sub> absorption.<sup>23</sup>

These forms of gastritis usually are asymptomatic.<sup>124</sup> The causes,<sup>115</sup> which are multiple and incompletely understood, may include (1) chemical, thermal, and mechanical injury to the mucosa, (2) nutritional deficiency (iron, folate, ascorbate), (3) endocrinologic insufficiency (thyroid, adrenal, pancreatic), (4) genetic abnormalities, and (5) autoimmune mechanisms, as discussed below.

The relation of the gastritis to pernicious anemia continues to be debated. Gastric biopsy in patients with pernicious anemia may demonstrate either gastric atrophy or severe atrophic gastritis.<sup>115</sup> Two possibilities must be considered. (1) that pernicious anemia represents the end-point of progressive gastritis, and (2) that the genetic lesion of pernicious anemia interacts with the gastric disorder to produce pernicious anemia. As noted above, both of these may be correct, leading to two types of pernicious anemia, "inherited" and "acquired."<sup>120</sup> According to a prospective study, simple atrophic gastritis did not appear to progress to overt pernicious anemia.<sup>138</sup>

## Immunologic Mechanisms

### Circulating Antibodies

In the sera of patients with pernicious anemia, several antibodies that react with human tissue antigens have been described

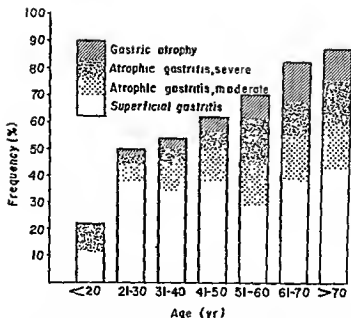


Fig 15-4 Prevalence of gastritis by age in the general population as determined by gastric biopsy (Prepared from the data of R A Joske<sup>121</sup>)

(Table 15-1). These have been called *auto-antibodies* because they appear to be directed against the tissues of the host. The most frequently found antibody reacts with antigens in the cytoplasm of *gastric parietal cells* and is detected by either a complement-fixation or immunofluorescent technique.<sup>111</sup> This antibody may also be found in a small proportion of apparently "normal" subjects (who probably have asymptomatic gastritis, see Fig. 15-4) and in about half of those known to have gastritis (Table 15-1).

*Antibodies to intrinsic factor* are detected less frequently, but are more specific since, with very rare exceptions,<sup>134</sup> they have been found only in pernicious anemia patients (Table 15-1). Intrinsic factor antibodies are of two types.<sup>136,137</sup> The more common are the so-called "blocking" antibodies, which prevent vitamin B<sub>12</sub> from complexing with intrinsic factor and are therefore presumed to react at or near the B<sub>12</sub> binding site. The less common, "binding" (or "precipitating") antibodies, which are rarely found in the absence of blocking antibodies, bind either intrinsic factor alone or the intrinsic factor-B<sub>12</sub> complex and presumably react with a part of the molecule not required for B<sub>12</sub> attachment. Intrinsic factor antibodies may be detected by

several techniques, including radioimmunoassay.<sup>135</sup> After intrinsic factor antibodies have been complexed with intrinsic factor, they may be separated from parietal-cell antibodies by gel filtration.<sup>122</sup> All of these serum antibodies belong to the IgG class of immunoglobulins. They are heterogeneous with respect to light-chain composition, indicating that they are not produced by a single clone of cells and probably represent a normal immunologic response to antigenic stimulation.<sup>108</sup>

Circulating cytotoxic antibodies directed against lymphocytes have also been found in pernicious anemia subjects.<sup>118</sup> Their significance is unknown, but they also occur in persons with other "autoimmune disorders" such as lupus erythematosus and rheumatoid arthritis.

Antibodies also have been found in the gastric juice,<sup>117,132</sup> where they may be either of the IgG or of the secretory IgA type, the latter indicating an origin within the stomach itself.<sup>117</sup> Formation of autoantibodies by cells within the gastric mucosa also was suggested by an immunofluorescence technique.<sup>107</sup> Gastric juice antibodies may be difficult to detect because they are often complexed with intrinsic factor.<sup>132</sup> They may occur in the

absence of a corresponding serum antibody.<sup>111,133</sup>

Also found in patients with pernicious anemia and their relatives are complement-fixing antibodies to thyroid acinar cytoplasm<sup>112</sup> (Table 15-1).

### Cellular Immunity

In addition to the humoral antibodies, phenomena typical of cell-mediated immune mechanisms may occur in pernicious anemia subjects. In one study, lymphocyte transformation was observed when lymphocytes from 3.4 to 37.5% (depending on the antigen used in the test) of patients with pernicious anemia were exposed to various gastric antigens.<sup>110</sup> In other studies, leukocyte migration has been inhibited by intrinsic factor or other gastric antigens in patients with overt pernicious anemia.<sup>112,131</sup>

### Association of Pernicious Anemia with Certain, Possibly "Autoimmune," Disorders

In patients with pernicious anemia the incidence of thyroid disease is comparatively high<sup>101</sup> (Table 15-2) and vitiligo also is common.<sup>121</sup> Diabetes is frequently associated with pernicious anemia, but, since diabetes is so common in the general population, a truly increased incidence in pernicious anemia subjects has been difficult to establish.<sup>23</sup> Two rare endocrinologic diseases have been reported in association with pernicious anemia often enough to suggest that the occurrence is greater than would be expected on the basis of chance alone; namely, Addison's adrenal atrophy (20 cases) and idiopathic hypoparathyroidism (17 cases).<sup>23</sup> In one study of 99 patients with pernicious anemia, the prevalence of rheumatoid arthritis (5 cases) was not significantly greater than in the control group; however, there was an increased prevalence (11 cases) of a positive reaction to the test for rheumatoid factor.<sup>113</sup> Coombs' positive hemolytic anemia has been observed infrequently.<sup>110,131</sup>

### Significance of the Autoimmune Phenomena

Whether the autoimmune manifestations in pernicious anemia are the result<sup>119</sup> or a cause<sup>23</sup> of the disease continues to be controversial.<sup>5</sup> It is possible that the inflammatory gastritis leads to release of tissue antigens followed by an appropriate antibody response. Also, the inflammation conceivably may alter normal tissue components so as to render them antigenic. Antibodies formed to such stimuli might not circulate at first because they would combine with the remaining normal antigens. With the antigen loss accompanying continued and progressive tissue destruction, the combining capacity would decrease, and measurable titers of the antibody would then appear. Thus the appearance in the circulation of intrinsic factor antibody, for example, might signify only that tissue levels of intrinsic factor were either lost or so depleted as to be insufficient to absorb the antibody. Such a sequence of events has its parallel in the appearance of antibodies to glomerular basement membrane after removal of kidneys afflicted with glomerulonephritis, and the disappearance of these antibodies when renal grafting is performed.<sup>5</sup>

Possible pathogenetic actions of the humoral and cellular antibodies include: (1) damage to cells and/or induction or aggravation of inflammatory gastritis and (2) inhibition of vitamin B<sub>12</sub> absorption. As yet, no inflammatory or cytotoxic activity has been demonstrated for either parietal-cell or intrinsic factor antibodies. However, it is possible that cell-mediated mechanisms are of greater importance in such reactions.<sup>112,130</sup> To some investigators, the histologic picture of the gastric lesion, especially the infiltration with lymphocytes and plasma cells, suggests immunologic inflammation and resembles that of the lesion of autoimmune (Hashimoto's) thyroiditis.<sup>111</sup>

Intrinsic factor antibodies may inhibit vitamin B<sub>12</sub> absorption if they are present in gastric juice,<sup>111</sup> but they appear to have little or no effect if they are confined to the circulation.<sup>125</sup> Thus, absorption of vitamin B<sub>12</sub>

**Table 15-1. Frequency of Autoantibodies in Patients with Pernicious Anemia and Other Disorders**<sup>23,111,123,134,143,144</sup>

Subjects		Frequency of Antibodies		
		to Parietal Cells	to Intrinsic Factor	to Thyroid Cytoplasm
Normal	Men under 55	5.6%	very rare	5.2%
	Men over 55	9.2%	<1%*	
	Women under 55	14.7%	very rare	12.2%
	Women over 55	22.3%	<1%*	17.8%
Patients with pernicious anemia		84%	56%	55%
Relatives of pernicious anemia patients		36%	6%*	50%
Patients with gastritis		47%	rare	rare
Patients with myxedema		32%	4%	87%
Relatives of patients with myxedema		20%	—	46%

\*Probably with latent pernicious anemia

given with excess hog intrinsic factor was normal (19.3%) in pernicious anemia patients lacking intrinsic factor antibodies, and was not significantly reduced in those with serum antibodies only. However, absorption was clearly reduced in patients with antibodies in gastric juice only or in those with antibodies in both gastric juice and serum (to 11.1% and 8.4%, respectively).<sup>133</sup> Further evidence for

an effect of gastric juice intrinsic factor antibody on absorption is the observation that porcine intrinsic factor is more effective than human intrinsic factor in facilitating vitamin B<sub>12</sub> absorption in patients with pernicious anemia.<sup>102</sup>

In three reports, the transplacental transfer of intrinsic factor antibodies was documented.<sup>106,110,116</sup> These studies presented an

**Table 15-2. Association between Pernicious Anemia and Certain Other Diseases**

Disease	Occurrence of Indicated Disease in		Occurrence of Pernicious Anemia in Patients with Indicated Disease
	Controls	Patients with Pernicious Anemia	
Diabetes mellitus	1.3–1.7%	2.4%	0.4%
Graves disease		1.8%	3.1%
Myxedema		2.4%	10.8%
Vitiligo	0.1%	1.6%	8% <sup>117</sup>

Combined data from a number of investigations, as summarized by Chanarin.<sup>23</sup>

opportunity to observe the effect of such antibodies on a normal infant. Intrinsic factor was present in the gastric juice of one of three infants and absent in the other two, one of whom became deficient in vitamin B<sub>12</sub> at age three months. All of 36 control infants were found to have intrinsic factor in their gastric juice. These observations suggest that a circulating antibody can sometimes, but not always, impair the secretion of gastric intrinsic factor.

Possibly bearing on the pathogenetic importance of the autoimmune phenomena is the effect of adrenal corticosteroids. These agents have been observed to bring about increased vitamin B<sub>12</sub> absorption and hematologic improvement in patients with pernicious anemia, but not in those with the megaloblastic anemia that follows gastrectomy.<sup>101,101</sup> This response sometimes, but not always, has been accompanied by histologic improvement in the gastric mucosa and by the appearance in gastric juice of hydrochloric acid and increased amounts of intrinsic factor. With steroid therapy, serum titers of intrinsic factor antibody decreased in 18 of 28 patients (64%)<sup>101</sup>; however, the enhanced vitamin B<sub>12</sub> absorption was observed both in patients with such serum antibodies and in those without. Furthermore, absorption often improved before any decrease in serum antibody titer was observed.<sup>103</sup> Few data are available regarding steroid-induced changes in gastric juice antibodies, in at least two subjects, such antibodies disappeared as vitamin B<sub>12</sub> absorption improved, but, in others, there was no demonstrated relation between the improved absorption and the presence or absence of gastric juice antibodies.<sup>101</sup> Thus, although effects on antibody synthesis or action seem the most attractive explanation for the influence of steroids, proof for this hypothesis is still wanting.

More than 20 patients have been described in whom pernicious anemia and adult-onset immunoglobulin deficiency coexisted.<sup>142</sup> In most of these patients, the immunoglobulin deficiency was apparent before the pernicious anemia was detected. The pernicious anemia

in these individuals was typical in every respect except for an early age of onset (mean, 34 years) and the absence of circulating antibodies to parietal cells, intrinsic factor, or thyroid tissue. This observation constitutes strong evidence that humoral antibodies are not necessary for the development of pernicious anemia. On the other hand, "auto-immune" disorders such as rheumatoid arthritis are relatively common in patients with immunoglobulin deficiency. In explanation of this apparent contradiction concerning the role of immunologic mechanisms in the pathogenesis of pernicious anemia, it has been suggested that cell-mediated immunologic mechanisms become exaggerated in immunoglobulin deficiency, and can then play a role in certain autoimmune phenomena, including the gastric atrophy of pernicious anemia.

## Clinical Manifestations

### Mode of Onset and Initial Symptoms

The onset of pernicious anemia is characteristically insidious, and by the time the patient seeks medical attention the anemia may be moderately severe. An average of 15 months was found to elapse from the onset of symptoms to diagnosis.<sup>207</sup> It is common to discover a degree of anemia that is much greater than suggested by the appearance of the patient or the severity of his symptoms. The chief exceptions to this rule are those patients in whom symptoms referable to the nervous system appear early and progress rapidly before anemia has become severe or has even developed. Also, soreness of the tongue may appear early, before anemia of an appreciable degree of severity is present.

The diagnostic triad of weakness, sore tongue, and paresthesias is the classic symptom complex at presentation, but the initial symptoms actually vary. Not infrequently they suggest the presence of some digestive disorder, or cardiac, renal, or genitourinary disease, or even mental aberration or obscure infection. In such instances, the underlying

**Table 15-3. Presenting Complaints in Pernicious Anemia<sup>207</sup>**

Symptoms of anemia	58%
Paresthesias ✓	13%
Gastrointestinal complaints ✓	11%
Sore tongue or mouth ✓	7%
Weight loss ✓	5%
Difficulty in walking ✓	3%
Other ✓	3%

disease may not be suspected until the blood has been examined.

Initial complaints, in order of frequency, are listed in Table 15-3. Some of these may be found to have been present for a number of months, rarely for several years and, characteristically, have varied in their intensity from one time to another.

#### Outward Appearance of the Patient

When the anemia is severe, the skin has a delicate lemon-yellow tint resulting from the combination of pallor and mild icterus. The sclerae may be yellowish, but often the increased bilirubinemia detectable in the blood is barely manifested clinically. There may be diffuse or blotchy, brownish pigmentation<sup>202</sup> interspersed with patches of vitiligo.<sup>121</sup> The skin may be dry, but is often peculiarly velvety and smooth, yet inelastic. Petechiae are unusual.

It is remarkable how frequently patients suffering from this disease have blond or prematurely gray hair and light-colored eyes.<sup>205</sup> Often the face is wide and the chest is broad with a wide costal angle; however, there are many exceptions to these generalizations.

Weight loss, while often slight, may be considerable. The average weight loss in a series of 50 patients was 14 pounds.<sup>226</sup> As a rule, the loss can be explained on the basis of anorexia.

#### Body Temperature

Fever of several degrees is common when the anemia is severe and may occur in the

absence of infection. Unexplained fever was found in 22% of patients in one series.<sup>208</sup> Such fever disappears promptly following effective treatment.

#### Gastrointestinal System

An abnormal tongue is found in about half the patients with pernicious anemia, and is one of the classic manifestations of the disease.<sup>23</sup> Tongue symptoms may occur in the absence of anemia. Their appearance or re-appearance in pernicious anemia patients portends a relapse and, in patients under treatment, is an indication of inadequate therapy.

When glossitis is at its height, the tongue is very painful and "beefy" red. Its entire dorsum may be involved, or there may be red patches at the margins or on the dorsum. Rarely the entire mouth and throat may be involved, causing the patient to complain of burning and pain on swallowing. More frequently, there is only burning or soreness, particularly of the anterior half of the tongue.

The intensity of the glossitis usually subsides after several days, but the soreness often recurs at intervals of varying length. Between attacks, the epithelium of the tongue is left devoid of papillae, thus producing the smooth, glazed tongue that is characteristic of pernicious anemia (Fig. 15-5). Partial loss of taste may ensue. Some patients whose tongues are quite smooth deny ever having had sore tongues. Some restoration of the papillae often is associated with adequate therapy.<sup>219</sup>

Loss of appetite is a frequent complaint. In one large series, 65% had "poor" appetite, and in 14% the symptom was so severe that all desire for food was lost and the diet consisted only of fluids and small amounts of breads and cereals.<sup>207</sup>

The frequency of diarrhea as a manifestation of pernicious anemia varies greatly (7% to 50% of cases), but, even when frank diarrhea has not occurred, direct questioning frequently will bring out the fact that the patient has a semisolid bowel movement immediately on arising and another one or two stools later



Fig 15 5 Smooth tongue of patient with pernicious anemia

in the day, a pattern possibly associated with achlorhydria. In one series, however, constipation was more common than diarrhea.<sup>207</sup>

Less frequently, complaints referred to the gastrointestinal tract may suggest the presence of a large variety of diseases, including gallbladder or malignant disease, or peptic ulcer. Pseudotumors caused by hypertrophy of the musculature of the pylorus have been described.<sup>203,218</sup> There may be anorexia, nausea, "gas," a sense of fullness and epigastric discomfort, heartburn, vomiting, and irregular abdominal pain that varies in intensity. Attacks of paroxysmal pain resembling the gastric crisis of *tuberculous dorsalis* sometimes occur.<sup>201</sup> Such an attack may be associated with vomiting and some abdominal rigidity. It is rare, however, to observe attacks as severe as are often seen in patients with sickle cell anemia. In pernicious anemia the pain may be symptomatic of changes in the spinal cord.

The liver may be slightly enlarged. When congestive heart failure is associated with the anemia, the liver may be greatly enlarged and tender. At autopsy the spleen is found to be moderately enlarged in practically all subjects, but there is no agreement as how often it is palpable clinically. In one large series,

it was palpable in 19% of patients<sup>207</sup>; in others, the incidence ranged from 3% to 40%.<sup>204</sup> Pronounced enlargement is unusual. With adequate treatment, the liver and spleen recede behind the costal borders.

### Circulatory System

The cardiovascular symptoms of anemia were discussed in Chapter 13. In pernicious anemia, they may be so pronounced as to lead to a mistaken diagnosis of primary cardiovascular disease. Dyspnea, palpitation, sensations of extra beats, excessive weakness, vertigo, and tinnitus may be associated with signs of congestive cardiac failure, including well-marked edema and cardiac and hepatic enlargement. In other patients angina pectoris may develop.<sup>220</sup> Systolic murmurs at the base of the heart and even at the apex are often heard. Occasionally, a diastolic murmur at the aortic area, possibly due to dilatation of the aortic ring, is detected. Cardiac irregularity seldom occurs but tachycardia is common. The vessels in the neck may be seen to pulsate if the anemia is severe and a loud venous "hum" may be present.

### Genitourinary System

Occasionally the clinical picture of chronic nephritis may be simulated by pernicious anemia. The renal function, as measured by fixation of the specific gravity of the urine, may be impaired and small amounts of albumin and casts may be found.<sup>206</sup> However, the nonprotein nitrogen, blood urea nitrogen, or creatinine values are not often elevated. The renal defects generally decrease with subsidence of anemia.

In patients with severe neurologic disease, impaired micturition and urinary retention may occur. This may predispose to infectious complications, such as cystitis.

Infertility has occasionally been ascribed to pernicious anemia.<sup>214</sup>

### Nervous System<sup>220</sup>

At one time, complaints referable to the nervous system were encountered in as many

as 95% of pernicious anemia patients, and the degree of involvement was considered severe in about 30%.<sup>213,228</sup> As facilities for diagnosis and treatment have improved, serious neurologic manifestations have become less common than they once were.<sup>203</sup> It is now estimated that about 30% of patients experience relatively mild neurologic disturbances (especially paresthesias and minor cerebral symptoms) and that signs and symptoms of spinal cord involvement are detected in only about 7%.<sup>23</sup>

The degree of nervous system involvement does not correlate well with the degree of anemia. In exceptional cases, pronounced neuropathy is found in the absence of any hematologic abnormality.<sup>222</sup> In one series, the blood hemoglobin concentration was greater than 9 g/dl in 30% of patients with distinct spinal cord involvement.<sup>207</sup> Neurologic disease may progress while the hematologic manifestations are corrected by folate ingestion.<sup>209</sup>

The neurologic illness chiefly affects the white matter of the dorsal and lateral columns of the spinal cord and the cerebral cortex. Peripheral nerve degeneration also occurs,<sup>217,225</sup> but it is uncertain whether this represents a distinct lesion or a process secondary to spinal cord disease. Because multiple nerve pathways are involved, the terms "subacute combined degeneration" and "combined system disease" have been applied.

With the possible exception of minor cerebral disturbances (see below), subjective sen-

sory complaints constitute the earliest and most frequent evidence of nervous system involvement<sup>231</sup> (Table 15-4). Most commonly, the patient experiences symmetrical tingling or "pins-and-needles" sensations, first in the tips of the toes and later in a stocking-and-glove distribution in all four limbs. Less frequently, the abnormal sensations may be described as "numbness," "coldness," or "tightness." Least common of all are tabes-like shooting pains. When paresthesias are the only symptom, there may be little or no objective evidence of neurologic disease.

At a later stage, there is clearer evidence of dorsal column involvement. The patient may complain of clumsiness and his gait may be incoordinated and ataxic. Reduced vibration sense, especially at higher frequencies (eg, 256 cycles/sec), is one of the earliest objective signs. Position sense may be impaired. The most sensitive way of detecting this abnormality is by manipulation of the index toe. The reaction to the Romberg test may become abnormal.

The lateral columns (pyramidal tracts) become involved in the later and more severe stages of the illness. Weakness and a spastic or "scissors" gait may develop. The Babinski sign is the earliest and most reliable objective evidence of lateral column disease. Hyperreflexia and clonus also may be observed.

If minor manifestations are included, cerebral disturbances may be considered to be common in patients with pernicious anemia

Table 15-4. Neurologic Manifestations in Pernicious Anemia

	Symptoms	Physical Findings	Location of Lesions
Mild	Paresthesias	None or slight impairment of touch and temperature sensation	Peripheral nerves(?) Dorsal columns
Moderate	Weakness Unsteady gait Clumsiness	Decreased vibration and position sense Romberg sign	Dorsal columns
Severe	Severe weakness Spasticity	Hyperreflexia Clonus Babinski sign	Dorsal and lateral columns



in relapse. The first manifestations may be cerebral<sup>221</sup> and may antedate other signs of the disease.<sup>211,230</sup> In a controlled study, depression and impaired memory were frequent, but only the latter seemed clearly related to vitamin B<sub>12</sub> deficiency.<sup>227</sup> In another study,<sup>221</sup> in 13 out of 17 patients with hemoglobin levels of 6.0 g/dl or less there was a clinically demonstrable reduction in the level of consciousness, justifying the diagnosis of delirium. In this study the most valuable single test was the serial subtraction of numbers, such as 7 from 100. Electroencephalograms demonstrated slowing in wave frequency. Therapy was associated with improvement in the level of awareness and in the behavioral disturbances consequent to the reduced awareness, and, in most instances, reversion of electroencephalographic tracing to normal. Since improvement was observed to take place about the time the reticulocyte response began, it seems likely that the cerebral involvement is a metabolic one and not secondary to the anemia.

More serious mental changes, as indicated by delusions, hallucinations, manic outbursts, and paranoid and schizophrenic states ("megaloblastic madness"), are uncommon.<sup>215</sup> Such psychoses may be curable if treated promptly.

Less common neurologic manifestations of pernicious anemia include ophthalmoplegia,<sup>212</sup> atony of the bladder, impotence,<sup>216</sup> perversion or loss of the senses of taste and smell,<sup>223</sup> and retrobulbar neuritis.<sup>214</sup> The last may be aggravated or precipitated by the use of tobacco.<sup>210</sup>

## Laboratory Findings

### Blood and Bone Marrow

The hematologic abnormalities in megaloblastic anemia were presented in detail in Chapter 14 and in Figures 14-1, 14-2, 14-3, 14-4, and 14-5. Pernicious anemia, the prototype of the megaloblastic anemia group, exhibits all of the abnormalities discussed in that section. The degree of the various deviations from normal varies with the severity of the deficiency.

Prior to the availability of specific therapy, unusually severe degrees of anemia were reported in patients with pernicious anemia. Naegeli found a red blood cell count of  $0.138 \times 10^{12}/l$  in one of his patients,<sup>259</sup> and Zadek recorded  $0.086 \times 10^{12}/l$ .<sup>260</sup> In Cabot's 1908 series, 21% of patients had red cell counts of less than  $1.0 \times 10^{12}/l$ , whereas only 1% of a series of 250 patients collected between 1944 and 1956 had a similar degree of anemia.<sup>208</sup> Today, most patients seek medical attention when the VPRC is between 0.15 and 0.25  $l/l$  (Fig. 14-2).

### Gastrointestinal Secretions

The total volume of gastric secretion is markedly reduced, eg, to an average of 15 ml/hr,<sup>254</sup> which is about 10% of normal. Furthermore, no increase in volume of secretion occurs after administration of histamine.

Absence of hydrogen ion secretion (achlorhydria) with maximum histamine stimulation (40  $\mu$ g/kg body weight) is practically a universal finding. Hydrogen ion secretion may be said to have occurred when the pH falls by at least one unit and to a value below 6.0 with such stimulation. In patients with pernicious anemia, basal pH values are always greater than 6.0 and usually greater than 7.0.<sup>23</sup> When histamine is given, the pH often remains the same or increases. In a small proportion of these patients an insignificant fall (0.1 to 0.7 units) may be observed. Earlier studies on gastric acid usually employed Topfer's reagent, by means of which the presence of "free acid" was inferred by a red color (pH value less than about 3.5). Obviously, by this relatively insensitive technique, no "free acid" should be found either before or after histamine stimulation.

Prior to 1951, 61 (of a total of 4215) patients who were said to have pernicious anemia with "free acid" in their gastric juice had been reported.<sup>258</sup> No such cases have been reported since more specific methods for establishing the diagnosis (eg, the Schilling test) have become available. It is probable that in many of these patients the megaloblastic anemia was due to folate deficiency or

vitamin B<sub>12</sub> malabsorption resulting from factors other than from lack of intrinsic factor.<sup>23,252</sup> In only one instance was a lack of intrinsic factor demonstrated by bioassay in a patient whose gastric secretions contained free acid.<sup>253</sup>

The hallmark of pernicious anemia is the extreme deficiency of intrinsic factor in the gastric secretion. An intrinsic factor assay suitable for routine clinical purposes is not presently available. When gastric juice was assayed by an immunologic method, the normal stomach was found to secrete 2000 to 18,000 units of intrinsic factor in the hour following histamine (40 µg/kg) stimulation. When 41 patients with pernicious anemia were studied by the same technique no intrinsic factor could be detected in 24 of them and less than 150 units were secreted by the others.<sup>23,251</sup> It was estimated that about 500 units are required for normal vitamin B<sub>12</sub> absorption. Intrinsic factor secretion also was reduced in a proportion of patients presumed to have atrophic gastritis without pernicious anemia, but usually the values were greater than those found in pernicious anemia patients. Peculiarly, patients with gastritis appeared to absorb more vitamin B<sub>12</sub> at a given level of intrinsic factor secretion than did pernicious anemia patients,<sup>251</sup> perhaps reflecting in the latter interference with absorption by intrinsic factor antibodies.

Gastric juice pepsin is reduced in pernicious anemia, but pepsin is not commonly measured because it has little more significance than free acid and is somewhat more difficult to quantitate. Similarly, pepsinogen in urine<sup>256</sup> or serum<sup>257</sup> tends to be reduced, but neither determination is a dependable test for pernicious anemia.

Findings on gastric biopsy were discussed earlier (page 606).

Pancreatic enzymes have been found to be present in patients with pernicious anemia, but tryptic activity was decreased, especially in those with moderate or advanced nervous system involvement.<sup>255</sup>

The diagnosis, differential diagnosis, and management were discussed in Chapter 14.

## Course, Prognosis, and Complications

Prior to the introduction of effective therapy the outcome in a case of pernicious anemia was fatal. The mortality rate in hospitalized patients was 53% in the first month.<sup>316</sup> However, in many patients the course of disease was one of remissions and relapses of varying severity and duration until death occurred, usually in one to three years.<sup>315</sup> Some patients were known to live for as long as 14 or even 20 years.<sup>313</sup> In Cabot's large series, remissions occurred in 86%, and 15% of the subjects had more than two remissions. Some of the remissions followed blood transfusion, the administration of arsenic, or splenectomy, and probably some patients unwittingly took liver or other substances now known to have real therapeutic value. Competent clinicians insisted,<sup>302</sup> however, that spontaneous remissions that were not the result of the inadvertent ingestion of antianemic material did occur in the pre-liver era. In any event, remission was rarely complete, and neurologic involvement progressed. In the few patients reported to have recovered, the diagnosis must be held in doubt.

Present methods of treatment have completely changed the outlook in pernicious anemia. Since 1926 there has been a striking decrease in mortality from pernicious anemia in various countries.<sup>303,312,314,315</sup> All ages and both sexes have shown lower mortality. Despite this improvement, the early mortality remains disturbingly high—14% in one recent series—largely due to cardiac failure occurring before a therapeutic response could be achieved.<sup>307</sup>

The most important present-day cause of relapse is the patient's reluctance to continue treatment for life and his readiness when new complaints develop to attribute them to the treatment. It is common to find that a relapse followed the onset of some intercurrent disease or that the need for some surgical measure distracted attention from antianemic therapy and caused it to be neglected at a time when it was most needed.

How soon relapse will occur once therapy has been stopped is unpredictable in the individual case. This depends not only on the development of intercurrent diseases but also, in addition to possible unknown factors, on the quantity of vitamin B<sub>12</sub> that is stored within the body, the inadvertent consumption of food factors having therapeutic value in pernicious anemia, and the degree of intrinsic factor deficiency. In one study of 54 cases,<sup>311</sup> relapses were found to develop at intervals varying from two to 38 months after therapy was discontinued. In a third of the subjects these appeared during the first six months and in 36% during the second six months. In 24% of the patients, relapse did not develop until the second year after treatment had ceased. Following withdrawal of liver extract in another group of 12 patients, six failed to show hematologic relapse in 26 to 29 months, and in the remainder relapse developed in eight to 18 months.<sup>306</sup> Remission lasted 42 to 78 months in four patients. In none did neurologic manifestations appear. It is probable that the time of recurrence of relapse will not always be the same even in the same patient.

The most serious long-term complication of pernicious anemia is carcinoma of the stomach. The apparent frequency of this complication varies with the method of detection. At autopsy, 5.2% of 2115 patients with pernicious anemia were found to have gastric carcinoma.<sup>23</sup> Combined studies based on clinical follow-up of living patients reveal that 2.3% of 8747 patients acquired this tumor.<sup>23</sup> This incidence is considered to be about three times that found in the general population.

The association of pernicious anemia with other hematologic disorders has been infrequent enough to have been the subject of case reports. Thirty-eight cases of association of pernicious anemia with leukemia (acute myeloblastic 14, chronic myelocytic 15, chronic lymphocytic 8, erythremic myelosis 1) have been reported.<sup>301,303,309,310</sup> In a prospective five-year study of 1625 patients with pernicious anemia, leukemia was found in three; the expected incidence in the general population would have been 0.89.<sup>301</sup> This increased

incidence of leukemia is of borderline statistical significance ( $p = 0.06$ ). At least 39 cases of polycythemia vera associated with pernicious anemia have been reported.<sup>23</sup> There are isolated cases of the coincidental occurrence of pernicious anemia and sickle cell trait<sup>304</sup> and erythrocytic hypoplasia.<sup>305</sup>

## Pathology

The morbid anatomic characteristics of pernicious anemia are now rarely encountered in the autopsy room. In the typical subject in relapse, the pathologist noted the yellowish appearance of the skin, the lack of wasting, and the dilated and flabby heart, as well as fatty changes in the parenchymatous viscera, especially the liver, heart, and kidneys. Heavy deposits of iron were found in the liver, spleen, and kidneys. The red bone marrow was found to be strikingly deepened in color and the yellow marrow of the long bones was transformed into a deep-red gelatinous substance that was likened to currant jelly. In the spleen and liver, foci of extramedullary blood formation were regularly found.

These changes are absent in patients dying as a result of intercurrent disease during vitamin B<sub>12</sub>-induced remission. In such patients, however, as well as in those dying in relapse, morphologic abnormalities are found in the *gastrointestinal tract*.

The mucosa of the tongue usually is atrophied. The changes in the stomach<sup>323,331</sup> are striking in tissue fixed soon after death. Severe atrophy of the upper two thirds of the stomach is evident even to the naked eye, this area being reduced to the thinness of parchment. At the junction of the body with the pyloric mucosa, the transition to the normal thickness of the stomach wall is usually abrupt. Microscopically, in the mucosa of the fundus and body of the stomach, the only structures seen are the surface epithelium and a few scattered glands lined by mucus-producing cells. The specialized oxyntic and peptic cells are not seen. However, there is no fibrosis, cellular infiltration, or other evidence of past inflammation. The muscle coat



Fig 15-6. Gastric columnar cells A, Normal. B, untreated pernicious anemia (Magnification 750 $\times$ ) (Courtesy of Dr Cyrus E Rubin)

likewise shows atrophy. No evidence of a reduction in the number of argentaffin cells, at one time proposed as the source of intrinsic factor, is found.<sup>329,331</sup> Findings on gastric biopsy were discussed previously (page 606). No reorganization of the mucosa follows intensive therapy.<sup>111,331</sup>

Examination of cells obtained from the stomach by gastric lavage and abrasion has revealed groups of columnar cells that differ from normal ones chiefly in size.<sup>332</sup> These cells are approximately twice the normal size, their nuclear membrane may be creased or folded in appearance, and small aggregates of chromatin stand out on the relatively empty background of nucleus. The cytoplasm, as well as the nucleus, is enlarged and has a more vacuolated and granular appearance than is normal (Fig. 15-6).

The nuclei of cells from the buccal mucosa are enlarged<sup>321</sup> and those from the vagina show atypical changes suggestive of neoplasia.<sup>325,330</sup>

Changes in the *central nervous system* vary in extent. Myelin degeneration and loss of

nerve fibers in the dorsal and lateral tracts of the cord, and degenerative changes in the dorsal root ganglia are commonly found. Following liver therapy an increase in gliosis has been reported.<sup>324</sup> Degenerate changes are not infrequent in peripheral nerves<sup>223,326,327</sup> and degeneration of the celiac ganglia and of Auerbach's and Meissner's plexuses as well as lesions in the lateral horns of the spinal cord have been described.<sup>322</sup> Changes similar to those found in the spinal cord have been observed in the brain.<sup>328</sup> The rarity of optic atrophy has been mentioned. Changes in other cranial nerves have not been described.

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## *Anemias Characterized by Deficient Hemoglobin Synthesis and Impaired Iron Metabolism*

Definition and Detection  
Etiology and Pathogenesis  
Laboratory Evaluation  
Diagnostic Approach

THE anemias to be discussed in this chapter and in Chapters 17 and 18 are grouped together because they share important pathogenetic features: quantitatively deficient hemoglobin synthesis in association with impaired iron utilization or actual iron deficiency. When such a defect is present, erythrocytes may be formed in normal numbers, but they contain less than the normal amount of hemoglobin. As a result, the anemia in these disorders is most accurately measured by the blood hemoglobin concentration, which is reduced to a greater extent than either the red cell count or the volume of packed red cells.

In contrast to other forms of anemia, the major morphologic alterations in this group of anemias, when they are fully developed, are hypochromia and microcytosis. However, these morphologic characteristics may not always be present, especially when the anemia is mild or of short duration. At first the anemia is normocytic and may then become

somewhat hypochromic. The anemia of chronic disorders almost never progresses beyond this. Even when there is iron deficiency, the anemia does not become hypochromic or microcytic until several months after iron stores have been depleted. The sideroblastic anemias often are characterized by two populations of cells, only one of which is hypochromic and microcytic.

### **Definition and Detection of Hypochromic, Microcytic Anemia**

*Microcytosis* refers to the presence in the blood of abnormally small cells. In the strictest sense, the term means that the *volume* of each such cell is reduced. Usually, but not always, other dimensions, such as diameter and thickness, are decreased in proportion to volume. Simply because of their small size, microcytes contain less than the normal amount of hemoglobin.

*Hypochromia* indicates the presence of erythrocytes in which the *concentration* of hemoglobin is reduced. Obviously, hypochromic cells may be of normal size and still contain reduced amounts of hemoglobin.

When the *average* cell contains less than



**Table 16-2. Pathogenetic Classification of the Anemias Characterized by Deficient Hemoglobin Synthesis**

- A Disorders of iron metabolism
  - 1 Iron deficiency anemia (Chapter 17)
  - 2 Anemia of chronic disorders (Chapter 18)
  - 3 Atransferrinemia
  - 4 The Shahidi Nathan Diamond syndrome
  - 5 Hereditary hypochromic anemia (sla) in mice
  - 6 Experimentally induced copper deficiency in swine (Chapter 4)
- B Disorders of globin synthesis
  - 1 The thalassemias (Chapter 26)
  - 2 Certain other abnormal hemoglobin diseases (Chapters 24 and 25)
- C Disorders of porphyrin and heme synthesis sideroblastic anemias (Chapter 18)
  - 1 Defective  $\delta$ -aminolevulinic acid (ALA) synthesis
    - a Vitamin B<sub>6</sub> deficiency
    - b Defective vitamin B<sub>6</sub> metabolism induced by drugs or toxins
    - c Defective ALA synthetase activity
  - 2 Deficiency of coproporphyrinogen oxidase
  - 3 Deficiency of heme synthetase
  - 4 Lead intoxication
  - 5 Of unknown cause

*transferrinemia*, plasma iron is low. Its specific transport protein is lacking; disease is extraordinarily rare, having been reported only three times.<sup>36,32a,37</sup> The disease appeared to have been inherited as an autosomal recessive trait, since trans-ferrin values in the subjects' parents were half normal.<sup>32a,37</sup> The patients suffered from severe, refractory, hypochromic, microcytic anemia from infancy. Iron absorption was impaired. Excessive amounts of iron were present in the hepatic reticuloendothelium, but little iron was found in the bone marrow. Values for serum iron and TIBC were low and ferrokinetic studies demonstrated that the ability of marrow normoblasts to utilize iron for hemoglobin synthesis was severely impaired. These abnormalities were corrected by the administration of iron.<sup>32a</sup> One child died of cardiac failure secondary to hemosiderosis.<sup>37</sup> Extremely rare is the disorder that Shahidi, Nathan, and Diamond described in two siblings, one male and one female.<sup>61</sup> In these children, the anemia was severely hypochromic and microcytic. The serum iron level was low and the transferrin was completely

saturated. Hepatic parenchymal cells contained grossly excessive amounts of iron, but none was found in hepatic or bone marrow RE cells. Similar findings were reported in another pair of siblings.<sup>62a</sup> The nature of the defect in these families remains unknown. Shahidi, Nathan, and Diamond suggested that there was an abnormality in RE function and that developing normoblasts became deficient in iron because they received none by direct transfer (ropheocytosis) from marrow RE cells. However, although such a transfer may indeed occur (Chapter 4), amounts of iron adequate for normal hemoglobin synthesis can be supplied by transferrin alone.<sup>42</sup> It is tempting to surmise that in these children there was a functional defect in the transfer of iron from transferrin to immature erythrocytes. However, such a defect was searched for and could not be found.

An X-linked gene (sla) gives rise to hereditary hypochromic anemia in mice.<sup>2</sup> These animals absorb iron poorly, as has been demonstrated in vivo as well as in studies with everted duodenal sacs.<sup>2,52</sup> However, defective iron absorption cannot fully explain the anemia, since affected mice become anemic in

ro despite normal placental iron transfer.<sup>20</sup> Furthermore, although the hemoglobin rises when iron is given parenterally, the response is not complete. Thus, additional defects in iron utilization probably exist in this animal model.

The hypochromic, microcytic anemia of copper-deficient swine has been discussed in chapter 4 (page 150).

### Disorders of Globin Synthesis

The thalassemias are a group of inherited disorders in which synthesis of one of the normal polypeptide chains of globin is severely deficient (Chapter 26). In the heterozygous state (thalassemia minor), hypochromia and microcytosis may be prominent, even though anemia is usually absent or mild. In more severe thalassemic disorders, including beta-thalassemia major and hemoglobin H disease, hypochromic, microcytic anemia may be severe.

In thalassemia, the cellular hemoglobin deficit comes about by two mechanisms. The first of these is the aforementioned defect in synthesis of one of the normal chains, for example, the beta chain in beta thalassemia. The second mechanism is related to the fate of the complementary normal chain. In beta thalassemia, excess alpha chains are formed. These, being poorly soluble, precipitate intracellularly forming inclusions resembling Heinz bodies.<sup>26</sup> The inclusions evidently can be removed by the normal spleen without the cell being destroyed. The result is a cell even more deficient in hemoglobin than would be predicted by the magnitude of the synthetic defect.

In most of the abnormal hemoglobin diseases, the hemoglobin is *qualitatively* abnormal, but synthesis is not impaired. In a few diseases, however, the anemia may be moderately microcytic. These include hemoglobin C disease<sup>62</sup> and hemoglobin E disease.<sup>14</sup> Pronounced hypochromia may sometimes be detected in unstable hemoglobin disease (Chapter 24) because heme may be lost when the abnormal hemoglobin precipitates intra-

cellularly and also because of Heinz-body removal.

### Sideroblastic Anemias

These are disorders which have in common the presence of ringed sideroblasts in the bone marrow,<sup>53</sup> a morphologic finding that indicates accumulation of iron in mitochondria.<sup>4</sup> The classification of all sideroblastic anemias as disorders of porphyrin and heme biosynthesis (Table 16-2) must be considered tentative since the pathogenesis is unknown in many instances.

A number of the sideroblastic anemias (pp. 678-689) result from defective synthesis of delta-aminolevulinic acid, the first step in porphyrin synthesis. This reaction requires the coenzyme form of vitamin B<sub>6</sub>, pyridoxal phosphate. Therefore, induced vitamin B<sub>6</sub> deficiency in animals leads to sideroblastic anemia.<sup>22,45</sup> Vitamin B<sub>6</sub> deficiency is rare in man, but lack of coenzyme B<sub>6</sub> can come about by other means. For example, ethanol<sup>39</sup> and certain antituberculosis drugs (isoniazid,<sup>50,66</sup> cycloserine,<sup>66</sup> pyrazinamide<sup>49</sup>) occasionally produce sideroblastic anemia by interfering with formation of pyridoxal phosphate from its precursors.<sup>39,48,60</sup> Again, some hereditary and acquired sideroblastic anemias respond to pharmacologic doses of vitamin B<sub>6</sub> and are therefore called pyridoxine-responsive anemias.<sup>35</sup> The exact nature of the defects in this heterogeneous group is not known, but at least some of them may represent abnormalities of the enzyme, aminolevulinic acid synthetase.<sup>45,67</sup>

There are other examples of sideroblastic anemia in which defective heme synthesis seems well established. A rare, hereditary anemia has been observed in which coproporphyrin oxidase appears to be defective.<sup>30,36</sup> A patient with heme synthetase deficiency has been reported.<sup>59</sup> Intoxication with lead inhibits several of the heme biosynthetic enzymes.<sup>32</sup> On the other hand, a defect in heme synthesis has not been documented in the relatively common acquired, idiopathic or secondary forms of sider-

oblastic anemia that do not respond to pyridoxine.<sup>40,41</sup>

## Laboratory Tests Useful in Detection and Differential Diagnosis

### Hematologic Observations

There usually is little difficulty in distinguishing typical, well-developed iron-deficiency anemia from the anemia of chronic disorders. Anemia, hypochromia, and microcytosis generally are much more pronounced in iron deficiency (Table 16-3) than in the chronic disorders, as are the degrees of anisocytosis and poikilocytosis. However, when the iron deficiency is early and mild, morphologic findings in the two diseases may be similar.

Thalassemia minor can usually be identified by finding an MCV that is unusually low for the mild degree of anemia (Table 16-3) and by the presence of apparent hypochromia (see above). A discriminatory function (DF)

has been developed that can distinguish iron deficiency from thalassemia trait with erythrocyte indices alone.<sup>25a</sup> It is calculated from the formula:

$$DF = MCV - RBC - (5 \times Hb) - 34$$

Positive values for DF indicate iron deficiency; negative values, thalassemia trait. In addition, basophilic stippling and target cells are more prominent in thalassemia than in iron deficiency. However, detection of these changes may require very careful scrutiny of the blood smear, since the changes may not be striking. There is rarely any problem in detecting thalassemia major, because the severe hypochromic, microcytic anemia is accompanied by signs of hemolysis, including reticulocytosis and increased plasma bilirubin. Furthermore, the blood smear is characterized by the presence of normoblasts, and marked anisocytosis and poikilocytosis, as well as by stippling and target cells.

Homozygous hemoglobinopathies are usually microcytic and normochromic, and there are many target cells in the blood smear.<sup>14,62</sup>

The various forms of sideroblastic anemia

**Table 16-3. Degree of Anemia, Microcytosis, and Hypochromia in Certain Anemias**

	Hb g/dl	MCV fl	MCHC g/dl
Normal	♂ 16 (14-18) ♀ 14 (12-16)	89 (83-96)	34 (32-36)
Iron-deficiency anemia <sup>1</sup>	7.5 (4-12)	74 (53-93)	28 (22-31)
Anemia of chronic disorders <sup>19</sup>	10.4 (8-13)	86 (70-95)	32 (26-36)
Thalassemia (Chapter 26)			
Minor	11.6 (9-14)	68 (56-75)	31 (29-33)
Major	(2-7)	(48-72)	(23-32)
Hemoglobin H <sup>41b</sup>	8.8 (7-11)	70 (53-88)	25 (24-28)
Abnormal hemoglobins			
CC <sup>42</sup>	10.4 (7-14)	74 (55-93)	32 (23-38)
EE <sup>14</sup>	10.5 (8-13)	69 (66-72)	32 (29-34)
Sideroblastic anemia			
Hereditary <sup>15</sup>	6.4 (4-10)	77 (49-104)	25 (14-30)
IRSA <sup>44</sup>	10.0 (7-12)	104 (83-118)	32 (26-36)

Values are means with approximate range in parentheses. Abbreviations: Hb, blood hemoglobin concentration; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; IRSA, idiopathic refractory sideroblastic anemia.

Table 16-4. The Blood Smear in Hypochromic, Microcytic Anemia

	Anisocytosis, Poikilocytosis	Basophilic Stippling	Target Cells	Dimorphism
Iron-deficiency anemia	1-3+	0	±	±
Anemia of chronic disorders	±	0	±	±
Thalassemia				
Minor	1+	2+	5%(0-37%)*	0
Major	3+	3+	3+	0
Hemoglobin C or E disease	2+	±	50%(10-90%)*	0
Sideroblastic anemia				
Hereditary	3+	2+	2+	3+
IRSA	1+	2+	±	3+

\*Expected % of target cells in smear

Abbreviations, 0, none, ±, may be present, 1+, present, 2+, easy to find 3+ prominent, IRSA, idiopathic refractory sideroblastic anemia

differ considerably in their hematologic manifestations. However, the two most typical features are erythrocyte dimorphism—a hypochromic population of cells mixed with a relatively normal population on smear—and the presence of occasional, heavily stippled, hypochromic cells. In hereditary (X-linked) sideroblastic anemia, the anemia, hypochromia, and microcytosis are pronounced (Table 16-3), and these changes are accompanied by considerable anisocytosis and poikilocytosis, with numerous target cells. In acquired, idiopathic, refractory sideroblastic anemia (IRSA), the hypochromic population is small; the MCV is usually increased and the MCHC is normal or slightly decreased.

## Iron Metabolism

### Serum Iron and Iron-Binding Capacity

The study of iron metabolism should begin with a determination of serum iron and total iron-binding capacity. The determinations are relatively simple, but certain precautions must be taken in collecting the specimen. The patient should have fasted for 12 hours and should not have consumed any iron-containing medication for at least 24, and preferably 72 hours. Specimens should be obtained in the morning because the normal diurnal variation results in low values later in the day.<sup>5</sup>

Syringes, needles, and sample containers must be free of iron; ordinary laboratory glassware is usually contaminated with iron unless specially processed, but most plastic ware is iron-free. Standard methods for preparing glassware and determining plasma iron colorimetrically have been recommended by an international committee.<sup>41</sup> Several methods are available for measuring TIBC,<sup>7,69,71</sup> which is an indirect measure of serum transferrin content.

From the serum iron and TIBC, transferrin saturation can be calculated by the following formula:

Transferrin saturation (%)

$$= \frac{\text{serum iron } (\mu\text{g/dl}) \times 100}{\text{TIBC } (\mu\text{g/dl})}$$

The normal value for transferrin saturation is 20 to 45%.

In both iron deficiency anemia and the anemia of chronic disorders, the value is reduced below 16% (Fig. 16-2).<sup>1</sup> The degree of reduction tends to be greater in iron deficiency than in chronic disorders, but there is considerable overlap between these two conditions. A value of less than 5% is almost certainly due to iron-deficiency anemia. In sideroblastic anemias, transferrin saturation is almost invariably increased and often approaches 100%.

The absolute value for TIBC may be help-

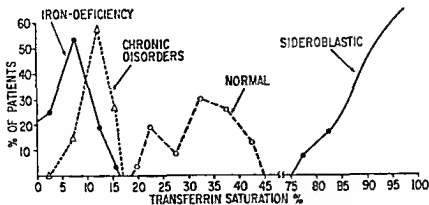


Fig 16-2. Transferrin iron saturation in patients with several types of anemia. The values are low (less than 16%) in both iron deficiency and the anemia of chronic disorders, with considerable overlap. Values of less than 5% are found only in iron deficiency. Transferrin saturation is increased in sideroblastic anemia.

ful in distinguishing between iron deficiency and the anemia of chronic disorders. Often, TIBC is increased in iron deficiency and decreased in chronic disease (Fig. 16-3). However, there is considerable overlap between values in the two groups. In iron deficiency a bimodal distribution of values for TIBC has been observed and, in a proportion of patients, the TIBC may be decreased (Fig. 16-3). When these patients are studied further, they are found to have a generalized hypoproteinemia, one indication of which is decreased serum albumin concentration.<sup>1</sup>

### Measurement of Iron Stores

In order to measure iron stores, it usually is necessary to sample one of the two principal storage depots, the bone marrow or the liver. Aspiration and biopsy<sup>46</sup> of marrow usually are preferred because they are probably safer and also the technique is more familiar to hematologists than liver biopsy.

If bone marrow aspirates are used, it is essential to be certain that there is an adequate number of marrow particles in the smear. Some prefer to assess thick smears that are unstained.<sup>54</sup> In such preparations, hemosiderin appears as golden-yellow, refractile granules. More often, the specimen is stained by the Prussian blue method,<sup>55</sup> which colors hemosiderin blue. With experi-

ence, the marrow hemosiderin stores can be graded from 0 to 6+ (Table 16-5 and Plate X).<sup>29</sup> Good correlation between histologic grading and iron content has been found.<sup>29,61</sup> Normal marrow is graded 1+ to 3+. In iron deficiency, marrow hemosiderin is absent; in

### TOTAL IRON-BINDING CAPACITY (TIBC) IN IRON DEFICIENCY

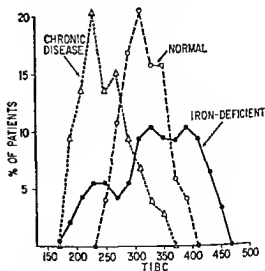
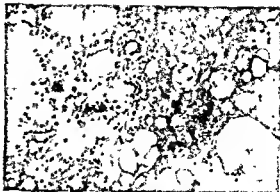


Fig 16-3. Total iron-binding capacity (TIBC) in patients with iron deficiency and in those with chronic disease. A high value indicates iron deficiency. Low values are found in chronic disease, but may also occur in iron deficiency.

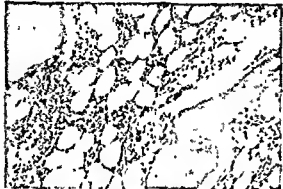
# PLATE X



A



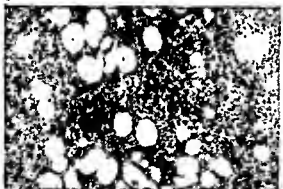
B



C



D



E



F



G



H



I



J

Iron stores in bone marrow ( $\times 100$ ). Also sideroblasts and siderocytes ( $\times 1000$ ). A-F, Bone marrow biopsies (A, C, E) and crush (aspiration) preparations (B, D, F), stained with Prussian blue for iron, showing normal bone marrow iron (A, B), absence of iron (C, D) and excessive iron stores (E, F). A, C, E counterstained with Giemsa. Sideroblasts are shown in G and H, the latter being 'ringed'. I and J are siderocytes.

Table 16-5. Criteria for Grading Iron Stains in Bone Marrow Aspirates<sup>29</sup>

Grade	Criteria	Iron Content ( $\mu\text{g/g}$ ) <sup>*</sup>
0	No iron granules observed	$43 \pm 23$
1+	Small granules in reticulum cells, seen only with oil immersion lens	$130 \pm 50$
2+	Few small granules visible with low-power lens	$223 \pm 75$
3+	Numerous small granules in all marrow particles	$406 \pm 131$
4+	Large granules in small clumps	$762 \pm 247$
5+	Dense large clumps of granules	$1618 \pm 464$
6+	Very large deposits obscuring marrow detail	$3681 \pm 1400$

<sup>\*</sup>M  $\pm$  SD

the anemia of chronic disorders, iron is always present, most often with a grade of 2 or 3+ but sometimes 4 or 5+.<sup>1</sup> In cases difficult to diagnosis, study of marrow hemosiderin is the most reliable way of distinguishing between iron deficiency and the anemia of chronic disorders. Iron stores are normal or moderately increased (2 to 4+) in thalassemia minor and in the homozygous hemoglobinopathies. They are greatly increased (5 to 6+) in thalassemia major and in sideroblastic anemias.

Iron stores can also be estimated by liver biopsy. Both histochemical<sup>51</sup> and chemical<sup>28</sup> methods of analysis have been described. Conventional needle biopsy techniques with Menghini or Vim-Silverman needles or a fine needle aspiration method<sup>47</sup> have been used. The latter procedure is said to be free of complications. Criteria for grading the preparations from 0 to 4+ have been published,<sup>28,47,51</sup> and these correlate well with chemical analysis.<sup>28</sup>

Storage iron also has been evaluated by urinary excretion of iron after the administration of a chelating agent.<sup>16,23,27,34,55</sup> The chelator most commonly employed for the purpose is deferoxamine (DFOM); diethylenetriaminopentaacetic acid (DTPA) is less satisfactory.<sup>34</sup> In the DFOM test, 500 mg of DFOM are given (im) and iron is measured in urine collected during the subsequent 24

hours. The more complicated differential ferroxamine test requires the injection of <sup>59</sup>Fe-labeled ferroxamine to correct for the fraction of chelate that is not excreted into the urine.<sup>27</sup> Little is gained by this added complexity.<sup>34</sup>

There is a rough correlation between iron stores and urinary iron excretion after DFOM. In one study, excretion in normal subjects averaged 0.7 mg, range 0.1 to 3.6; in iron deficient subjects, excretion was 0.1 mg, range 0 to 1.7; and in sideroblastic anemia, 7.8 to 15.5 mg.<sup>34</sup> As these data imply, the test is useful in detecting iron overload. It is less reliable in detecting deficient stores because the values found in normal and in iron-deficient subjects overlap.<sup>34,55</sup> The chelatable iron probably is a measure of parenchymal cell iron rather than total stores,<sup>34</sup> although a contrary view has been expressed.<sup>16</sup>

A novel in vivo method of estimating iron stores, based on the magnetic susceptibility of ferritin and hemosiderin, has been described.<sup>3</sup> A large, semicircular transformer was constructed which included an air gap between the primary and secondary windings. The magnetic properties of specimens placed in this gap affected electron flow in the secondary winding. When the body of a rat was moved slowly through the gap and scanned for magnetic susceptibility, values obtained correlated well with chemical liver analysis.

As yet, apparatus suitable for human use has not been constructed.

According to one study,<sup>41a</sup> the status of iron stores can be estimated by measuring the ferritin in serum. With a radioimmunologic assay, the mean serum ferritin concentration was 69  $\mu\text{g/l}$  in men and 35  $\mu\text{g/l}$  in women. The range was wide in both sexes, but levels below 10  $\mu\text{g/l}$  were associated with iron deficiency. Values greater than 1000  $\mu\text{g/l}$  were common in iron overload states, including sideroblastic anemia.

### *The Cobalt Excretion Test*<sup>65</sup>

The gastrointestinal absorption of cobalt correlates closely with the absorption of iron. Unlike iron, however, much of the absorbed cobalt is excreted into the urine. These properties of cobalt form the basis for the cobalt excretion test, which may be considered to be an indirect measure of iron absorption. In this test, cobalt (20  $\mu\text{moles}$  and 0.5  $\mu\text{Ci}$  in 100 ml 0.01 N HCl) is given by mouth, and the urinary excretion of the isotope is measured. Normal subjects over 30 years of age excreted an average of 7.3% (range, 4 to 11%) into the urine in a six-hour period.<sup>65</sup> In younger subjects, age 15 to 30, slightly higher values were found, mean 10% (range 5 to 14%). In iron deficiency, cobalt excretion was increased to a mean of 18% (range 12 to 26%) in patients over 30 years of age and to 19% (range 15 to 27%) in patients 15 to 30 years of age. Thus, the test clearly distinguishes iron-deficient subjects from normal persons. Since the results become abnormal as soon as iron stores are depleted, the test may provide one of the earliest and most sensitive laboratory means for detecting iron deficiency. Values for the cobalt excretion test fell within normal limits in 30 patients with a miscellaneous group of anemias not due to iron deficiency, including several patients with the anemia of chronic disorders and one with thalassemia minor. However, cobalt excretion is increased in idiopathic hemochromatosis, and also in other situations in which iron absorption may be increased in the absence of iron deficiency, such as acute

blood loss and hemolytic anemia. Since iron absorption may be increased in thalassemia major and in sideroblastic anemia, it is likely that cobalt excretion will be increased in these conditions as well.

### *Sideroblasts and Siderocytes*

Nucleated red cells that contain stainable iron granules are known as sideroblasts. These cells can be observed and counted in marrow aspirations stained with the Prussian blue technique and counterstained with saffranin.<sup>10</sup> In the experience of some investigators, the staining procedure must be carried out at 56° C when sideroblasts are to be counted,<sup>1,24</sup> although others have not found this to be necessary.<sup>10</sup>

Usually, the number of cells containing iron granules is recorded as percent of all the normoblasts present. It is useful to note the number and size of the granules, since more than five small granules in each normoblast are considered abnormal.<sup>24</sup> It also is most important to note the presence of so-called ringed sideroblasts.<sup>6</sup> These are normoblasts in which five or more granules form a partial or complete ring about the nucleus (Plate X). It is rare to find even one such cell in normal marrow.

In normal subjects, 30 to 50% of the red cell precursors are sideroblasts. Both in normal subjects and in patients with a variety of anemias, there is excellent correlation between transferrin saturation and the proportion of sideroblasts<sup>1</sup> (Fig. 16-4). However, a great deal of experience in counting sideroblasts is required to reproduce this correlation. Marrow sideroblasts are decreased to less than 10% (average 2.5%) in iron deficiency. They are also decreased, but to a lesser degree (6 to 20%, mean 13%), in the anemia of chronic disorders. In these situations, marrow sideroblasts offer no more information than does the value for transferrin saturation.

The most important application of sideroblast counts is in making the diagnosis of sideroblastic anemia. This group of anemias is characterized by excessive accumulation of iron within the mitochondria of normo-



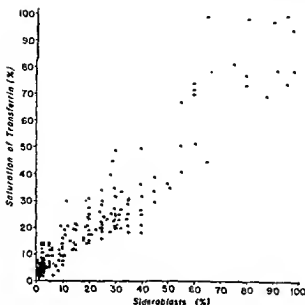


Fig 16-4 Relation between marrow sideroblast count and transferrin saturation (From Bainton and Finch,<sup>1</sup> courtesy of the authors and American Journal of Medicine)

blasts.<sup>4</sup> Mitochondria characteristically are distributed about the nucleus and the presence of iron granules in a similar distribution suggests that the iron is in the mitochondria. Admittedly, electron microscopy is required to determine the presence of mitochondrial iron deposition with certainty, but for clinical purposes the detection of ringed sideroblasts is adequate evidence of this. In sideroblastic anemia, most of the normoblasts are sideroblasts, but, more specifically, many of them are ringed.<sup>53</sup>

The enumeration of *siderocytes* (circulating erythrocytes containing iron granules) yields less valuable information, probably because the spleen removes siderotic granules shortly after the red cells have entered the circulation. Of 11 patients with sideroblastic anemia, only six had increased numbers of circulating siderocytes.<sup>43</sup> The number of siderocytes was also increased in patients with severe hemolytic anemia and in those who had been splenectomized or had splenic atrophy.

#### Free Erythrocyte Protoporphyrin

Under normal circumstances, red cell precursors synthesize slightly more proto-

porphyrin than is needed for heme synthesis. The excess remains with the cell and is called free erythrocyte protoporphyrin (FEP). The normal concentration varies somewhat with the method and the laboratory, but composite normal limits are approximately from 15 to 80  $\mu\text{g/dl}$  red cells.<sup>70</sup> Although known to be a sensitive indicator of disorders of heme synthesis, the FEP has been used only rarely for clinical purposes because methods for its determination have been exceedingly tedious and time-consuming.<sup>70</sup> A new, simplified method that makes possible the analysis of one specimen in 30 minutes or 12 in 2 hours has been described.<sup>38</sup> Such a method should bring the determination within the reach of the routine clinical laboratory.

FEP increases approximately fivefold in iron-deficiency anemia.<sup>13,19,36</sup> Furthermore, it is one of the earliest and most sensitive biochemical abnormalities associated with this disorder.<sup>41a</sup> It was found to be elevated in 13 of 15 patients with latent iron deficiency (ie, deficient stores but no anemia).<sup>19</sup> FEP increases in the anemia of chronic disorders to a degree similar to that found in iron-deficiency anemia.<sup>9,41a</sup> The increase appears to be better related to the duration than to the severity of the anemia.

FEP was found to be increased in thalassemia in one study,<sup>36</sup> but not in others.<sup>31,58,63</sup>

Changes in FEP in sideroblastic anemia vary with the nature of the lesion. In vitamin B<sub>6</sub>-deficient swine, FEP was reduced and the value returned to normal after administration of pyridoxine.<sup>12</sup> A similar response has been observed in pyridoxine-responsive anemia in man.<sup>57,67</sup> One variety of hereditary sideroblastic anemia is characterized by very low values for FEP combined with high values for erythrocyte coproporphyrin, and is thought to represent a defect in the coproporphyrinogen oxidase reaction.<sup>30,36</sup> In contrast, FEP is usually increased in idiopathic refractory sideroblastic anemia, and becomes further increased with pyridoxine therapy.<sup>44</sup> In one patient, whose illness was thought to represent a defect in the heme synthetase reaction, FEP was elevated to extremely high levels,

1750 µg/dl, and this was associated with dermal photosensitivity.<sup>59</sup>

In lead poisoning, FEP is increased and other abnormalities in porphyrin metabolism also are observed. These include excessive excretion of delta-aminolevulinic acid and coproporphyrin into the urine.<sup>32</sup>

### Globin

Methods used in evaluating the protein portion of the hemoglobin molecule are discussed in detail in Chapter 24. Beta-thalassemia minor may be detected by an increase in the proportion of hemoglobins A<sub>2</sub><sup>31</sup> or F.<sup>15</sup> In contrast, the value for hemoglobin A<sub>2</sub> decreases in iron deficiency.<sup>68</sup> In beta-thalassemia major, hemoglobin F usually is greatly increased.

Alpha-thalassemia minor may be detected

## THE HYPOCHROMIC, MICROCYTIC ANEMIAS

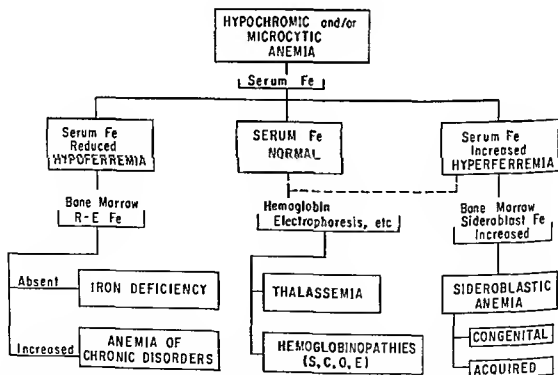


Fig. 16-5 Flow sheet for diagnosis of hypochromic, microcytic anemia. The dashed line is meant to indicate that hyperferremia may be found in thalassemia.

by the presence of hemoglobin Barts ( $\gamma_4$ ) at birth or of hemoglobin H inclusion bodies in older individuals,<sup>41b</sup> but the hemoglobin often is completely normal in older children and adults. Hemoglobins C, E, and H may be detected by electrophoresis; unstable hemoglobins, by the heat-denaturation test (Chapter 24).

## Diagnostic Approach

A tentative diagnosis usually is possible after the initial history has been obtained and the physical examination and hematologic evaluation have been completed. History-taking should be directed toward ascertaining whether the condition is congenital, as it is in thalassemia and other hemoglobinopathies and in hereditary sideroblastic anemias, or acquired, as it is in iron deficiency, the anemia of chronic disorders, and acquired sideroblastic anemia. A careful evaluation for blood loss and other etiologic factors in iron deficiency should be made (Chapter 17). Possible diseases associated with the anemia of chronic disorders, such as rheumatoid arthritis, infection, and carcinoma, should be searched for. This information, together with the hematologic data discussed previously, often is adequate to establish the diagnosis.

When the diagnosis remains obscure, or when confirmation of the clinical impression is needed, the steps indicated in Figure 16-5 should be followed. An accurate determination of serum iron permits classification into one of three categories (Figs. 16-2 and 16-5). When the serum iron is reduced (transferrin saturation less than 16%), only iron deficiency and the anemia of chronic disorders need be considered and these can be distinguished by estimation of iron stores as judged by bone marrow examination, or, possibly, by performing the cobalt excretion test. High transferrin saturation suggests sideroblastic anemia, and its presence can be confirmed by examination of the bone marrow after staining for iron. The amount of iron in the serum in patients with thalassemia or the hemoglobinopathies usually is normal

but may be increased. Diagnosis is based on family studies and analysis of the hemoglobin (see Chapters 24 and 26.)

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## Iron Deficiency and Iron-Deficiency Anemia.

- History
- Iron-Deficiency States
- Prevalence\*
- Etiology
  - Diet
  - Impaired absorption
  - Blood loss
  - Infancy
  - Pregnancy and lactation
- Clinical Manifestations
- Laboratory Findings
  - The blood
  - Bone marrow
  - Kinetic studies
  - Iron metabolism
  - Iron enzymes
- Management

### History

The therapeutic use of iron was mentioned in Greek mythology in the story of Iphylus, who was cured of impotence by drinking iron rust dissolved in wine. Much of the iron therapy used by ancient physicians had its origin in such sympathetic magic, the sufferer hoping to assume something of the strength of steel by drinking water or wine in which a sword had rusted. The specific use of iron salts is credited to Sydenham who, in the early 1700's, recommended iron for treatment of chlorosis.

*Chlorosis*, a term derived from the Greek

word meaning green, was applied by Varandaeus<sup>21</sup> to a disorder which was first described in 1554 by Johannes Lange who called it "De morbo virgineo."<sup>20</sup> The disease became well known, not only in medical circles, but also to the laity, who called it the "green-sickness." It was depicted in many paintings by the Dutch masters and was alluded to by Shakespeare, Izaak Walton, and other literary figures of the period.<sup>15</sup> Chlorosis occurred almost exclusively in adolescent girls between the ages of 14 and 17. The most prominent manifestation was the greenish pallor which perhaps required "the eye of faith" to discern.<sup>6</sup> Other clinical features were breathlessness, palpitations, slight ankle edema, and gastrointestinal complaints, including perversion of the appetite, flatulence, abdominal pain, and constipation. Emotional disturbances ("love sickness"), depression, irritability, and moodiness were usual. Thrombophlebitis was a relatively common complication, and there was a particularly striking incidence of cerebral sinus thromboses.

It is clear that iron-deficiency anemia was a prominent part of the picture of chlorosis. In the 1830's, anemia, hypochromia, and lack of iron in the blood were detected by Hoefer, Popp, and Foedisch, respectively,<sup>15</sup> and Ashwell was able to classify chlorosis as a disease of the blood.<sup>1</sup> In 1832, Pierre Blaud described the response of chlorosis to his de-

servedly famous pills (ferrous sulfate plus potassium carbonate). He gave gradually increasing doses, from 2 pills on the first day to 12 on the sixteenth day. Many excellent observers, including Niemeyer and Osler, confirmed his findings.<sup>16</sup>

As knowledge of iron metabolism has grown, most present-day investigators have come to view chlorosis as resulting from a combination of factors affecting adolescent girls: the demands of growth and the onset of menses, an inadequate diet, and a legacy of poor iron stores at birth from an iron-deficient mother. Others have pointed out that it is difficult to reconcile this concept with observations on the fluctuations in the incidence of the disease.<sup>23</sup> Chlorosis became especially common in the last decade of the 19th century. It then abruptly declined in incidence until only rare cases were observed after 1910. It is perhaps true that the disappearance of chlorosis has been exaggerated, since even today iron deficiency of moderate degree is by no means uncommon in girls and young women. Nevertheless, clinicians whose observations spanned the decades between 1890 and 1920 were astounded at the abrupt disappearance of a disease that had filled the benches of their waiting rooms and a large proportion of their hospital beds.<sup>24</sup> The most prominent hypothesis offered to explain the changing incidence of chlorosis relates the disease to tight lacing of the body with a corset or similar garment, a hypothesis that continues to attract advocates.<sup>21,23</sup> The case for this cause of chlorosis rests chiefly on the relation between the use of the corset and the incidence of the disease throughout the previous centuries.<sup>17</sup> Lacing as an adjunct to female fashion appears to have been introduced in the 16th century near the time of Lange's initial description of chlorosis. The corset was abandoned by most women, especially young women, near the turn of the century when the incidence of chlorosis markedly decreased. Furthermore, a decrease in the incidence of chlorosis probably accompanied the temporary disuse of the corset in the period from 1790 to about 1820. Although these time relations are impressive,

the theory has not been generally accepted because of failure to demonstrate a clear pathogenetic relation between lacing of the body and the development of iron deficiency. Extreme degrees of lacing compress the liver and lead to considerable displacement of abdominal organs (visceroptosis); however, there is no known way in which such changes lead to either defective absorption or excessive loss of iron.

A second form of iron deficiency which attracted considerable attention in the first three decades of the 20th century initially was called "idiopathic" or "primary" hypochromic anemia but was later termed "chronic hypochromic" anemia as its pathogenesis was clarified. This disorder differs in no important respect from iron-deficiency anemia as we know it today. Like chlorosis, chronic hypochromic anemia was found to be a disease chiefly affecting women; it differed from chlorosis in that it was detected later in life, especially in the fourth and fifth decades.<sup>27</sup> Other distinguishing clinical features were widespread epithelial changes involving the tongue and nails, as well as achlorhydria, which Witts believed to be of specific differential diagnostic value.<sup>28</sup> The anemia was most common among women with poor diets, multiple pregnancies, and menstrual irregularities. Although it is clear in retrospect that chronic hypochromic anemia responded to iron, at one time there was considerable disagreement on this point. In the period 1890 to 1920, iron therapy became discredited chiefly because of Bunge's claim that inorganic iron is valueless and that only organic preparations should be used, but also because von Noorden taught that no more than 0.1 g of metallic iron is necessary.<sup>16</sup> Furthermore, physicians failed to distinguish between iron-deficiency anemias and anemias due to other causes.

## Iron-Deficiency States

*Iron deficiency* is used to designate a condition in which the total body iron content has been depleted, no matter what the cause. The designation should not be taken to mean that

iron depletion has necessarily come about through nutritional deficiency. To avoid any such implication, the alternative term *iron-lack* has been proposed<sup>18</sup> but has not found general acceptance.

Since body stores of iron must be exhausted before red cell production is restricted, anemia is a late stage of iron deficiency. In the mildest stage, *prelatent iron deficiency*, the reticuloendothelial iron stores are subnormal but there is no biochemical evidence of deficiency.<sup>5,7,19</sup> The only physiologic consequence of prelatent deficiency is a compensatory increase in the rate of iron absorption,<sup>4,19</sup> an alteration which may be detected by means of the cobalt excretion test (Chapter 16, page 630).

*Latent iron deficiency* may be said to exist when iron stores are exhausted, but the blood hemoglobin level remains above the lower limit of normal.<sup>5,7,9,12,14,22,25,26</sup> In this stage, certain biochemical abnormalities in iron metabolism are usually detected, such as an increase in free erythrocyte protoporphyrin and a reduced plasma iron level.<sup>9</sup> There may also be less than normal urinary iron excretion following an injection of desferrioxamine B,<sup>14,21</sup> a decrease in tissue cytochrome oxidase levels,<sup>9</sup> and, much less regularly, a reduced value for the MCHC.<sup>22</sup> One study suggested that latent iron deficiency is

symptomatic,<sup>3</sup> but others have not.<sup>9,10,13</sup>

*Iron-deficient erythropoiesis* refers to any situation in which red cell production is limited by the plasma iron level.<sup>2</sup> Such a limitation regularly occurs when transferrin saturation falls below 16%.<sup>2</sup> Iron-deficient erythropoiesis does not always indicate iron deficiency since it also accompanies the reticuloendothelial iron block found in the anemia of chronic disorders (Chapter 18). As a consequence of iron-deficient erythropoiesis, the blood hemoglobin level falls, and newly formed red cells contain reduced amounts of hemoglobin.

An experiment in which normal volunteers were depleted of iron by phlebotomy demonstrated that, early in the course, most of the circulating red cells were normal, and erythrocyte indices were therefore not altered; however, a few abnormal cells were found in blood smear.<sup>8</sup> In time, microcytosis became apparent, and both the MCV and the MCHC were reduced. At this stage, the total iron-binding capacity increased. Still later in the course, the MCHC fell below normal.<sup>8</sup> Tissue iron levels probably do not become reduced to a level at which abnormalities of epithelium are observed until the deficiency is of very long duration. This progression forms the basis for the stages of iron deficiency outlined in Table 17-1.

Table 17-1. Stages of Iron Deficiency

Stage	R-E* Iron Stores	Plasma Iron	Anemia	Hypochromia, Microcytosis	Other Features
Normal	Normal	Normal	None	None	..
Prelatent deficiency	Reduced	Normal	None	None	Increased iron absorption
Latent deficiency	Absent	Reduced	None	Usually none	Increased FEP*
Early iron-deficiency anemia	Absent	Reduced	Mild to moderate	In some cells indices normal	...
Late iron-deficiency anemia	Absent	Reduced	Severe	Severe Reduced MCV, MCHC	Epithelial changes

\*FEP free erythrocyte protoporphyrin, R-E reticuloendothelial

Dr B. J. S. ...  
R. J. ...  
CHAPTER 17

## Prevalence

Iron deficiency probably is the most common form of nutritional deficiency, in both developing and developed countries.<sup>46</sup> It is reported to be the most common cause of anemia, both in general medical practice<sup>35</sup> and in the practice of clinical hematology<sup>32,41</sup> and is even alleged to be the most common organic disorder seen in clinical medicine.<sup>34</sup>

Despite these assertions, there are relatively few adequate studies of the prevalence of iron deficiency in various populations. The most commonly employed screening procedure has been a simple blood hemoglobin determination. As measured by this test, anemia was defined by the World Health Organization (WHO) as a value below 14 g/dl in men, 12 g/dl in nonpregnant women, and 11 g/dl in pregnant women.<sup>46</sup> Such a screening method cannot, by definition, detect *latent* iron deficiency. Furthermore, inaccuracies are introduced by anemias due to causes other than iron deficiency. Finally, hemoglobin values in "anemic" and "normal" populations overlap; a more sophisticated statistical analysis of the distribution curve is required in order to determine true prevalence.<sup>31</sup> Screening by determination of plasma iron and iron-binding capacity obviates some of these inaccuracies, but the procedure is more cumbersome and consequently has been performed less frequently. Still less suited for the study of large groups is determination of reticuloendothelial iron stores by bone marrow aspiration.<sup>45</sup> The response of a test population to iron medication is a widely applicable procedure.<sup>37</sup> Probably the most important source of error in population surveys is the selection of samples. Most reported series are heavily weighted with representatives of low economic groups, a bias which may increase apparent prevalence.

A representative group of population studies appears in Table 17-2. In most developed countries, about 3% of adult men, 20% of adult women, and over 50% of pregnant women are deficient in iron, as judged by plasma iron levels. Still higher figures are

found in countries such as India where malnutrition is widespread. The prevalence of iron deficiency in the United States has not been well studied.<sup>7</sup> Available data are based only on blood hemoglobin values (Table 17-2), and the criteria for abnormal levels have not always been those accepted by WHO and used in other studies. Furthermore, there has been little selection of samples to delineate social, economic, and geographic influences.

A still greater prevalence of iron deficiency would presumably be detected if iron stores were evaluated. A study of 114 healthy, white, nulliparous, socioeconomically privileged girls demonstrated that 24% of them had no storage iron and that an additional 42% had suboptimal stores.<sup>45</sup>

Iron deficiency is also common among children, especially during the first two years of life. Hypoferremia (transferrin saturation less than 15%) was found in about 30% of a carefully selected sample of 3423 preschool children from multiple geographic locations in the United States.<sup>43</sup> The incidence of hypoferremia was greatest (47%) in children 12 to 24 months of age, decreasing to 21% in five- to six-year olds. In the 12- to 24-month age group, hypoferremia was most common (62%) in children from lower socioeconomic groups, but prevalence was remarkably high (39%) even in children of upper middle class families.

## Etiology

Iron deficiency comes about either as a late manifestation of prolonged negative iron balance or because of failure to meet an increased physiologic need for iron. The normal mechanisms for maintaining iron balance are discussed in Chapter 4. Factors leading to negative iron balance or to increased requirements are listed in Table 17-3 and illustrated in Figure 17-1.

## Diet

The normal daily iron requirement for adult men is 5 to 10 mg and for adult women,



Table 17-2. Prevalence of Iron Deficiency in Various Populations

Country	Adult Men			Adult Women			Pregnant Women		
	Prevalence			Prevalence			Prevalence		
	n	Hypofer-remia* %	Anemia† %	n	Hypofer-remia* %	Anemia‡ %	n	Hypofer-remia* %	Anemia§ %
Israel <sup>44</sup>	66	9	14	100	11	29	100	46	47
India (Vellore) <sup>46</sup>	99	5	6	100	42	35	100	99	56
India (Delhi) <sup>44</sup>	—	—	—	95	26	64	100	52	80
Mexico <sup>46</sup>	111	3.6	0.9	110	28	12	124	61	27
Venezuela <sup>44</sup>	52	0	1.9	107	19	15	95	60	37
Latin America <sup>31</sup>	304	3	4	485	21	17	899	48	38
Wales <sup>40</sup>	499	6	—	517	22	—	—	—	—
Scotland <sup>42</sup>	—	—	—	500	21	8	—	—	—
Sweden <sup>37</sup>	—	—	—	414	—	171	—	—	—
USA <sup>31</sup>	165,408	—	0.81	73,783	—	12.6	—	—	—
USA, south, rural <sup>36</sup>	—	—	—	—	—	—	1699	—	53
USA, south, low-income, Negro <sup>41</sup>	—	—	—	—	—	—	4015	—	50
USA, Indianapolis <sup>37</sup>	—	—	—	—	—	—	4744	—	20
USA, San Francisco <sup>30</sup>	—	—	—	—	—	—	325	—	15

\*Transferrin saturation less than 15%

†Blood hemoglobin less than 14 g/dl

‡Blood hemoglobin less than 12 g/dl

§Blood hemoglobin less than 11 g/dl

|| Blood hemoglobin less than 12.3 g/dl

¶ Iron-responsive (Garby<sup>37</sup>)

n Number of persons surveyed

7 to 20 mg.<sup>7</sup> The amount of iron in the diet bears a rough relation to caloric content; in the United States the diet contains about 6 mg iron per 1000 calories. From these data it may be estimated that the average man consumes more iron than he needs but that many sedentary women subsist on an iron intake that is at best marginal. Thus, fortification of the female diet appears justified. Currently, in the United States about 2.7 mg iron are added to 100 g flour (in Great Britain, 1.6 mg/100 g), but such small amounts have little effect on total intake.<sup>104</sup> Higher levels of food supplementation (9.0 mg/100 g) have been proposed<sup>52</sup> but have not yet gained complete acceptance because it is unknown whether they would increase the risk of iron overload in men.<sup>69</sup>

Because of their larger iron stores and

comparative adequacy of their diets, iron deficiency is almost never seen in American males as a result of diet alone. Exceptions to this rule are so rare as to justify the reporting of a single case.<sup>127</sup> No doubt, the inadequacy of female diets partially explains the frequency with which latent deficiency occurs in women. Even in women, however, some etiologic factor in addition to poor diet usually is necessary before overt anemia develops.<sup>89</sup>

The situation is different in countries where poverty,<sup>72</sup> war,<sup>73</sup> unwise agricultural practices,<sup>123</sup> or religious and social tenets<sup>141</sup> have led to unusually deficient diets. The average diet of a group of women of the poor classes in Scotland was found to supply only 2.5 mg of iron per day. In Norway, where the diet supplies only about 4 mg/1000 calo-

**Table 17-3. Etiologic Factors in Iron Deficiency and Their Relative Frequency**

	Frequency* (%)
A Negative iron balance	
1 Decreased iron intake	
a Inadequate diet	19
b Impaired absorption	
1) Achlorhydria	41
2) Gastric surgery	10
3) Celiac disease	6
4) Severe iron deficiency (children)†	—
5) Pica	—
2 Increased iron loss	
a Gastrointestinal bleeding	56
1) Site unknown	16
2) Hemorrhoids	10
3) Salicylate ingestion	8
4) Peptic ulcer	7
5) Hiatal hernia	7
6) Diverticulosis	4
7) Neoplasm	2
8) Ulcerative colitis	1
9) Hookworm	—†
10) Milk allergy in infants	—
11) Meckel's diverticulum	—
b Excessive menstrual flow	29
c Blood donation	—
d Hemoglobinuria	—
e Self-induced bleeding	—
f Idiopathic pulmonary hemosiderosis	—
Goodpasture's syndrome	—
g Hereditary hemorrhagic telangiectasia	1
h Disorders of hemostasis	—
3 Cause unknown ("idiopathic hypochromic anemia")	17
B Increased requirements	
1 Infancy†	—
2 Pregnancy	6
3 Lactation†	—

\*Frequency figures based on 371 adult British patients with iron-deficiency anemia.<sup>49</sup> Figures total more than 100% because in many patients more than one factor was involved.

†In endemic areas 33%.<sup>124</sup>

grown in it were low in iron content,<sup>50</sup> hypochromic anemia was found to be common.

The role of diet and other factors in the development of iron deficiency in infants is discussed on page 645.

### Impaired Absorption

Histamine-fast *achlorhydria* is common in iron-deficient subjects (Table 17-3), but this finding may be a result of the deficiency as well as a factor in its development.<sup>96</sup> In Chapter 4, it was pointed out that gastric acid facilitates absorption of ferric iron and food iron, but has little effect on heme iron or ferrous iron.

Iron-deficiency anemia is a frequent complication following *gastric operations*, including total gastrectomy,<sup>51</sup> partial gastrectomy,<sup>53,54,90,92,111</sup> and vagotomy with gastroenterostomy.<sup>68</sup> Reduction in gastric acidity is only one factor in the impaired iron absorption that follows such operations. Other gastric secretions essential to iron absorption may be lost.<sup>113</sup> Also, since the most active sites of iron absorption are in the duodenum, the rapid intestinal transit that follows loss of the reservoir function of the stomach may lead to decreased absorption. For the same reason, iron deficiency is more common when the duodenum is surgically bypassed, as in Billroth II or Polya procedures, than when the normal channel is preserved.<sup>53</sup> Finally, recurrent bleeding as well as increased sloughing of iron-containing epithelial cells may contribute to the development of postgastrectomy anemia.<sup>138</sup>

In addition to gastrectomy, other defects in the gastrointestinal tract may lead to malabsorption of iron and contribute to the development of iron deficiency. Thus, the anemia associated with adult *celiac disease* (gluten sensitivity, sprue, idiopathic steatorrhea) is often hypochromic rather than megaloblastic;<sup>60,83,101</sup> in fact, iron-deficiency anemia may be the initial and dominant manifestation of celiac disease, with steatorrhea detectable only by laboratory studies.<sup>101</sup> Although iron absorption is usually increased in iron-deficient subjects, severe defi-

rites, a high frequency of anemia in males between 15 and 19 and above 50 years of age and in women from maturity until menopause was attributed to iron deficiency.<sup>115</sup> In regions where the soil and the vegetables

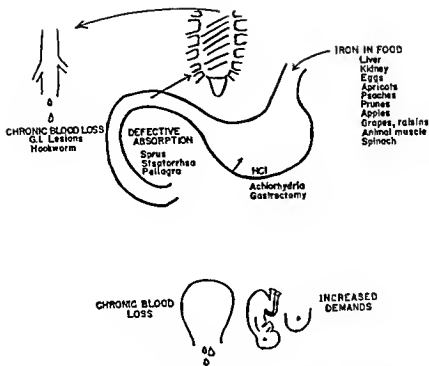


Fig 17-1. Factors concerned in the development of iron-deficiency anemia

ciency in infants was found to be associated with impaired iron absorption.<sup>102</sup> A similar defect could be induced experimentally in weanling dogs. The defect, which was corrected by iron repletion, was thought to result from reduced activity of mucosal iron enzymes such as cytochrome oxidase. Malabsorption of other nutrients, such as fat and D-xylose, may also occur in severely iron-deficient infants.<sup>84</sup>

*Pica* refers to the habitual ingestion of unusual substances, the most common of which is earth or clay (geophagia). Laundry starch (amylophagia)<sup>80</sup> and ice (pagophagia)<sup>86</sup> are others. Although pica may be a manifestation of iron deficiency and may be relieved when the condition is treated,<sup>105</sup> among certain cultural groups pica is practiced compulsively for reasons rooted in custom, folklore, or superstition.<sup>67</sup> In the United States the habit appears to be particularly common among Negro women, especially when pregnant, in the South and West, but also in New York City.<sup>126</sup> It is also practiced by natives of Turkey,<sup>116,129</sup> Egypt,<sup>120</sup> and Iran.<sup>120</sup> Iron deficiency is a common complication of both

clay-eating and starch-eating.<sup>80,109,126</sup> Clay can behave in the gut as an ion exchange resin and interfere with iron absorption. Normal subjects, who absorbed 27% of a 5-mg iron dose, absorbed only 2% when Turkish clay was ingested with the iron. Similarly, iron-deficient subjects absorbed 46% of the iron, and only 6% with Turkish clay ingestion.<sup>110</sup> Other clays also inhibited absorption, but to a lesser degree. Laundry starch does not interfere directly with iron absorption<sup>80</sup>; however, it is an almost pure carbohydrate with a very low iron content. When it is consumed in large quantities to the exclusion of other foods, an extremely deficient diet results.<sup>126</sup> In Iran, Egypt, and Turkey, geophagia has been implicated in a syndrome consisting of iron-deficiency anemia, hepatosplenomegaly, hypogonadism, and dwarfism.<sup>120,129</sup> Such patients probably are deficient in zinc as well as iron.

### Blood Loss

Blood loss is by far the most important cause of iron-deficiency anemia. It is impor-

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ries, a high frequency of anemia in males between 15 and 19 and above 50 years of age and in women from maturity until menopause was attributed to iron deficiency.<sup>115</sup> In regions where the soil and the vegetables

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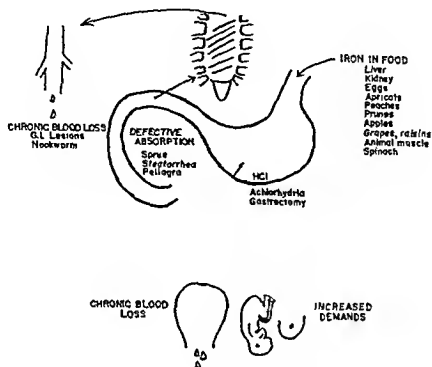


Fig 17-1 Factors concerned in the development of iron-deficiency anemia

ciency in infants was found to be associated with impaired iron absorption.<sup>102</sup> A similar defect could be induced experimentally in weanling dogs. The defect, which was corrected by iron repletion, was thought to result from reduced activity of mucosal iron enzymes such as cytochrome oxidase. Malabsorption of other nutrients, such as fat and D-xylose, may also occur in severely iron-deficient infants.<sup>84</sup>

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#### Blood Loss

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tant not only because of its frequency (Table 17-3), but also because the accurate detection, precise diagnosis, and proper management of the bleeding lesion may be of far greater importance to the ultimate well-being of the patient than repletion of his iron stores. For the purpose of estimating the effect of blood loss on iron balance, 1.0 ml of blood may be considered to contain about 0.5 mg iron. Thus, if an average diet is consumed, a steady blood loss of as little as 3 to 4 ml/day (1.5 to 2 mg iron) can result in negative iron balance.

### Gastrointestinal Bleeding

Gastrointestinal bleeding is by far the most common cause of iron deficiency in adult men and is second only to excessive menstrual blood loss as a cause in women. Any hemorrhagic lesion of the alimentary tract may cause iron deficiency. Those most likely to do so are the kind that are associated with occult bleeding or with the steady loss of small amounts of blood. Such lesions may go unnoticed or may be tolerated until the symptoms of anemia supervene. The relative frequency of the bleeding lesions leading to iron deficiency in a series of 317 adults<sup>60,144</sup> is given in Table 17-3. The list is not meant to be comprehensive since many other, less common lesions have been reported in association with isolated cases of iron-deficiency anemia.

*Hemorrhoids* are certainly one of mankind's commonest ailments, affecting more than a quarter of the population.<sup>94</sup> Two thirds of patients with hemorrhoids experience rectal bleeding, usually of a kind that is obvious to the patient since the blood streaks the outside of the stool or stains the water in the toilet bowl. Nevertheless, in one group of over 20,000 patients,<sup>62</sup> 80% allowed a year to elapse before seeking medical attention, and 32% had known of their disease for more than 10 years. A proportion of these patients become accustomed to the periodic loss of small amounts of blood, and gradual depletion of their iron stores occurs without their becoming unduly alarmed. Despite the frequency

with which hemorrhoids are associated with iron-deficiency anemia (Table 17-3), the clinician should be reluctant to accept them as the only bleeding lesions until a careful investigation has been made; hemorrhoids may obscure another, less obvious lesion elsewhere in the alimentary tract.

*Duodenal and gastric ulcers* are by far the most common cause of upper gastrointestinal bleeding,<sup>117</sup> which may be massive or occult. About 25% of patients with bleeding ulcers give no history of prior symptoms. When 1000 patients with ulcers were followed prospectively, 449 of them had 652 episodes of hemorrhage in 66,580 patient-months.<sup>117</sup> About half of these episodes of bleeding did not require blood transfusion and, therefore, were of the pattern that may result in iron depletion.

Although *aspirin* is considered by most laymen to be an innocuous drug, the frequency with which it causes blood loss is becoming apparent.<sup>128</sup> At a daily dose of 2 to 6 g of aspirin, 70% of patients were detected to have gastrointestinal bleeding at an average rate of 5 ml/day.<sup>132</sup> After seven days' treatment with only 2 aspirin tablets per day, or an equivalent dose of other salicylate-containing medications, excessive blood loss (1 to 4.5 ml/day) was detected.<sup>57</sup> The salicylate-induced lesion is probably an erosive gastritis.<sup>128,139</sup>

Iron-deficiency anemia occurs in about 15% of patients with *esophageal hiatal hernia*.<sup>143</sup> Anemia is particularly common (30%) with the paraesophageal variety and is more common when the hernias are large. Anemia may be the presenting manifestation and may occur even when there are no dyspeptic symptoms.<sup>93,143</sup> The average daily blood loss in 19 anemic patients with hiatal hernia was found to be 15 ml.<sup>93</sup> The reason for the bleeding remains controversial; reflux esophagitis is one possibility, but trauma to the gastric mucosa at the neck of the hernial sac is a more likely one.<sup>143</sup>

Although bleeding from colonic diverticula was once considered unusual, more recent studies have demonstrated a 5 to 8% incidence of hemorrhage due to *diverticulosis* and

15 to 25% due to diverticulitis.<sup>98</sup> Usually the blood loss is of mild degree and intermittent, and may resemble the pattern of hemorrhoidal bleeding. As with the latter, a careful investigation for other sources of intestinal blood loss must be made in order to exclude the possibility of neoplasm.

Iron-deficiency anemia may be the first sign of a neoplasm of the gastrointestinal tract, and the anemia may lead to its discovery in an operable stage. Carcinoma of the cecum often is clinically silent until anemia occurs. Less frequently, in carcinomas of other parts of the colon as well as of the stomach and the ampulla of Vater, iron-deficiency anemia may be the only initial symptom.

A survey of 32 patients with ulcerative colitis found 26 of them (81%) to have iron-deficiency anemia.<sup>95</sup> Average fecal blood loss of 6 to 25 ml per day was noted in five patients who were moderately anemic with relatively mild symptoms of colitis.

An important source of gastrointestinal blood loss in tropical areas is infection with the hookworm, *Necator americanus* or *Ancylostoma duodenale*.<sup>124</sup> Hookworm affects some 20% of the world's population.<sup>144</sup> The infection is endemic in a zone extending from the southern United States to northern Argentina in the Western Hemisphere, as well as in Mediterranean countries, South Asia, and Africa. The worms attach to the upper small intestine and suck blood from the host. The amount of blood lost is proportional to the number of worms harbored, which in turn can be estimated by the fecal excretion of hookworm eggs. On the average, each worm accounts for the loss of about 0.05 ml blood per day.<sup>81,123</sup> Female subjects harboring more than 100 worms (5 ml/day blood loss) and male subjects harboring more than 250 (12.5 ml/day blood loss) tend to become anemic.<sup>124</sup> The daily blood loss may be as great as 250 ml. Other factors affecting iron balance will determine whether the mildly infected individual becomes anemic. Deficient diet, repeated pregnancies, and achlorhydria have been found to be contributory factors in studies of hookworm anemia made

in Puerto Rico<sup>123</sup> and in Brazil.<sup>70,119</sup> It is significant that, among hookworm carriers in Argentina, those who consumed much meat were not anemic. In an endemic zone in Venezuela, 30% of noninfected individuals were anemic as compared with 46% of infected individuals.<sup>124</sup> Thus, the anemia in 16% could reasonably be attributed to the parasite.

At one time it was widely believed that hookworm anemia is due to elaboration of a toxin. No such mysterious concept is necessary. The anemia possesses all the characteristics of iron deficiency and can be relieved with iron therapy whether the worms are removed or not.<sup>70</sup>

Causes of gastrointestinal blood loss which are unique to infancy are discussed in a later section of this chapter (page 648).

### Reproductive Tract

Excessive menstrual blood loss is the most common single cause of iron deficiency in women (Table 17-3). In healthy, hematologically normal women, menstrual blood flow averages about 35 ml per menstrual period, and the upper limit of normal is about 80 ml per period.<sup>85,95</sup> Although flow varies considerably among different women, it is remarkably constant from one period to the next in the same individual.<sup>85</sup> In Swedish women with a dietary intake of about 10 mg iron per day, 67% of women with menstrual blood loss exceeding 80 ml/period were anemic.<sup>85</sup> In another study, British women with iron-deficiency anemia were found to lose 85 ml per period.<sup>95</sup> In contrast, however, a better nourished Canadian population tolerated flows greater than 80 ml per period without overt anemia or hypoferrremia.<sup>96</sup>

It is difficult to estimate menstrual blood loss by history, and many women are poor judges of the normality of their flow. Forty-one percent of women with blood loss exceeding 80 ml considered their periods to be "moderate" or even "scanty," and conversely 14% of women with flows less than 20 ml considered theirs to be "heavy."<sup>85</sup> Even medically sophisticated women may lose

more than 200 ml without realizing that their periods are unusual.<sup>112</sup> Thus, an adequate medical history in an anemic woman must contain a detailed description of the events during each menstrual period. Any of the following are indications of excessive menstrual flow: (1) inability to control flow with tampons alone, (2) use of more than 12 pads per period or four per day, unless the patient is unusually fastidious, (3) passage of clots, especially if they are larger than about 2 cm in diameter or if they persist after the first day, and (4) period duration exceeding seven days.

### *Blood Donation*

Regular blood donation may be an important source of iron loss. Each "unit" of blood donated contains about 250 mg of iron, often enough to exhaust the stores of a "normal" woman. Three or four such donations would exhaust the stores of a normal man. At one time, it was not unusual to encounter patients with iron-deficiency anemia caused only by frequent blood donations.<sup>54</sup> With precautions taken by present-day blood banks, such occurrences are unlikely. Still, blood donation would be expected to precipitate anemia in a patient with latent deficiency.

### *Hemoglobinuria*

Unnary iron losses averaging 1.8 to 7.8 mg/day accompany paroxysmal nocturnal hemoglobinuria.<sup>87</sup> Consequently, this rare disorder (Chapter 29) often is complicated by hypoferrremia and hypochromic anemia.<sup>87,100</sup> Hemoglobinuria in other disorders, such as the erythrocyte fragmentation syndromes associated with prosthetic cardiac valves (Chapter 28), also may be complicated by iron deficiency.<sup>122,123</sup>

### *Factitious Anemia*

An unusual cause of iron deficiency is self-induced blood-letting.<sup>59,71</sup> Almost all the instances of such anemia have been reported in unmarried women in paramedical occupa-

tions. Blood was removed by venipuncture, or bleeding was induced by injuring preexisting hemorrhoids or by laceration of the gastrointestinal tract with such instruments as knitting needles, or by means which remained obscure. In general, the women with this form of blood loss were described as hyperactive, obsessive, and intelligent and as displaying unusual hostility or contempt toward the medical profession.

### *Pulmonary Iron Sequestration*

Idiopathic pulmonary hemosiderosis<sup>75,108,134</sup> is characterized by repeated episodes of multifocal hemorrhages from alveolar capillaries. Iron in the shed blood is converted to hemosiderin by pulmonary macrophages, but cannot be reutilized for hemoglobin synthesis.<sup>108</sup> As a result, there is a steady sequestration of the body iron in the lungs, and iron-deficiency anemia almost invariably accompanies the disease. Frequently, anemia is the initial and only symptom. Other manifestations include cough, transient pulmonary infiltrates, and, after several years, pulmonary fibrosis, dyspnea, and digital clubbing. Pulmonary hemorrhage with hemosiderosis and iron-deficiency anemia also are common findings in Goodpasture's syndrome.<sup>58</sup>

### *Hereditary Hemorrhagic Telangiectasia*

This uncommon disorder is characterized by recurrent hemorrhages from the nose, gastrointestinal tract, and other sites (Chapter 36). Iron-deficiency anemia, sometimes very difficult to control, is an important complication of the illness.

### *Disorders of Hemostasis*

These illnesses rarely cause chronic blood loss leading to iron deficiency. As a rule the bleeding is acute, and treatment often involves replacement of blood as well as the coagulation factors which are lacking (Chapters 37 and 38).



### Idiopathic Hypochromic Anemia

The frequency with which iron-deficiency anemia occurs in the absence of a documented etiologic factor depends to some extent on the thoroughness of the investigation. No cause could be found at initial evaluation in 92 of 371 patients with iron-deficiency anemia.<sup>60</sup> However, a source of blood loss was later found in 28, leaving 64 (17%) to whom the label "idiopathic hypochromic anemia" could reasonably be applied. In this group the age, sex, and clinical manifestations were no different than in the patients with known causes. Thus, there is little justification to regard this group as representing a distinct entity. Instead, their underlying disease should be considered to be either in remission or not detectable at the time of examination.<sup>60,144</sup>

### Infancy

Some of the etiologic factors leading to iron deficiency in infancy are unique to this period of life and deserve special consideration (Table 17-4).

#### Decreased Total Body Iron at Birth

Body iron concentration at birth averages 78 mg per kg body weight (range 65 to 90 mg/kg) of which about 60 mg are accounted for as circulating hemoglobin and the rest is in stores.<sup>99</sup> Similar concentrations are found throughout fetal development, so that there is a roughly linear relation between body iron and body weight. Newborn babies in the

upper range of normal birth weights have 80% more iron than those in the lower range.<sup>63</sup> In a series of 272 infants the most important single factor predisposing to iron deficiency was *low birth weight*.<sup>145</sup> The incidence of severe anemia in infants whose birth weights exceeded 4000 g was extremely low whereas 80% of infants with a blood hemoglobin less than 5 g/dl were premature or weighed less than 3000 g at birth. In another study, 26.5% of a group of premature infants developed anemia with a blood hemoglobin of less than 9.0 g/dl.<sup>131</sup> The incidence dropped to 6.4% when feeding formulas were supplemented with iron.

Iron deficiency is more common in *twins* than in infants born singly. One factor is the tendency of twins to be smaller at birth. Another is the occurrence of placental transfusion of one monozygotic twin to another. Such an event can lead to a marked difference between the blood hemoglobin values of twins.<sup>82,121</sup> In a study of 130 sets of monozygotic twins, serious twin transfusion occurred in 19 (15%). In only four of these did both twins survive.<sup>121</sup> The late occurrence of iron deficiency in one member of a set of twins might represent a milder form of the same phenomenon.<sup>145</sup>

One of the important influences on iron in the newborn is easily controlled by the physician, namely, the time at which the umbilical cord is clamped at delivery. As much as 100 ml of fetal blood may remain in the placenta with early clamping of the cord.<sup>140</sup> Cord clamping delayed for only three minutes can result in a 58% increase in red cell volume.<sup>149</sup> Although the newborn has no immediate need for these erythrocytes the iron they contain can later be utilized to meet the demands of growth.

The influence of *maternal iron deficiency* on the body iron of the newborn is controversial, but the preponderance of evidence suggests that the depletion of maternal iron has little or no effect. In one study, infants born to mothers with blood hemoglobin concentrations less than 7 g/dl were found to have normal hemoglobin levels at birth, but tended to become anemic toward the end

**Table 17-4. Etiology of Iron Deficiency in Infancy**

- |   |                                    |
|---|------------------------------------|
| A | Decreased total body iron at birth |
| 1 | Low birth weight, prematurity      |
| 2 | Twins                              |
| 3 | Early clamping of umbilical cord   |
| 4 | Maternal iron deficiency           |
| 5 | Fetomaternal hemorrhage            |
| B | Growth                             |
| C | Inadequate diet                    |
| D | Blood loss                         |

of the first year of life.<sup>136</sup> Control groups of infants born to nonanemic or iron-treated mothers did not become anemic. In this study, environmental factors may not have been fully controlled; the mothers who became anemic may have been drawn from lower socioeconomic groups and may have fed their offspring an iron-deficient diet.<sup>61,103</sup> In another study, however, the red cell mass of infants born to severely anemic mothers was found to be 19% below that of a normal group.<sup>133</sup> In contrast, other investigators have found no difference between the hemoglobin values in infants of anemic and in those of nonanemic mothers either at birth or later.<sup>103,146</sup> Furthermore, iron supplementation during pregnancy had no effect on the subsequent development of iron deficiency in the infant.<sup>103,137</sup> These observations are consistent with studies made in animals which demonstrate that iron is transported across the placenta against a gradient, even at the expense of maternal iron stores.<sup>79,107</sup>

**FETOMATERNAL TRANSFUSION.** The transplacental passage of fetal erythrocytes into the maternal circulation is a physiologic event.<sup>65,106</sup> In about half of a series of 223 pregnant women, small amounts (mean, 0.4 ml) of fetal blood could be identified in the circulation between 21 and 42 weeks of gestation.<sup>106</sup> In the immediate postpartum period, fetal erythrocytes also were demonstrated in 50% of mothers; in 10% the fetal losses were "large" (0.5 to 40 ml) and in 1% they were "massive" (greater than 80 ml), usually resulting in overt anemia in the newborn.<sup>65</sup> In several instances, fetomaternal transfusion, probably over a prolonged time interval, has resulted in iron deficiency with hypochromic, microcytic anemia in the newborn.<sup>78,83,118</sup> In other cases, massive fetomaternal hemorrhage near term has led to hemorrhagic anemia, shock, or hydrops fetalis in the affected newborns.<sup>106,118</sup> It is likely that milder degrees of hemorrhage lead to reduced iron reserves in a proportion of newborns, thereby increasing the probability of the later development of anemia; however, the frequency of such occurrences cannot be estimated from presently available data.

## Growth

In the absence of disease, requirements of an adult male for iron vary little. In infancy, childhood, and adolescence, on the other hand, because of the increased needs of rapidly growing tissues, the requirements for iron are relatively great. The most rapid relative growth rates in human development occur in the first year of life. During this period, body weight and blood volume approximately triple, and the circulating hemoglobin mass nearly doubles (Table 17-5).<sup>131</sup> Still greater relative growth occurs in premature infants and others with low birth weights. A premature weighing 1.5 kg may increase his weight and blood volume sixfold, and may triple the circulating hemoglobin mass in one year (Table 17-5). If this rapid expansion of circulating hemoglobin is to occur, a large amount of iron is required. It has been aptly stated that "the infant bleeds into his own increasing blood volume."<sup>89</sup>

The relatively slower rates of growth in the ages from 1 to 12 years require a positive iron balance of about 0.2 to 0.3 mg per day. The growth spurt that occurs between 11 and 14 years of age requires a positive balance of about 0.5 mg/day in girls and 0.6 mg/day in boys.<sup>7</sup> Toward the end of this period the onset of menstruation occurs in girls, and their requirements then become those of adult women. The effects of age on iron requirements are shown in Figure 17-2.

## The Diet in Infancy and Childhood

Since unsupplemented milk is a poor source of iron (about 0.075 mg Fe/100 g milk),<sup>63</sup> most infants receive little iron in their diet until solid foods are introduced. Of the latter, the most important are iron-enriched cereals, which supply about 80% of the average infant's dietary iron during the first six months of life.<sup>64</sup> Recommended dietary iron intake for infants during the first year of life is about 6 mg per day<sup>63</sup> or 1.5 mg/kg body weight per day.<sup>7,131</sup> These are the highest requirements in relation to food intake that occur throughout life and are difficult to achieve without iron supplements,

Table 17-5. Iron Balance during the First Year of Life\*

	Full Term Infant		Premature Infant	
	Birth	1 Year	Birth	1 Year
Weight (kg)	3.3	10.5	1.5	9.5
Blood hemoglobin (g/dl)	20.0	12.3	20.0	12.3
Blood volume (ml)	290	800	135	720
Total hemoglobin (g)	58	98	27	89
Hemoglobin Iron (mg)	198	335	90	300
Storage & Tissue Iron (mg)	60	73	27	67
Total Body Iron (mg)	258	408	117	367
Net positive iron balance (mg/yr)	150		250	
iron balance (mg/day)	0.4		0.7	

\*Modified from Schulman<sup>131</sup>

especially if solid foods are introduced relatively late. Dietary requirements fall to about 1 mg/kg when the child is one year of age and 0.5 mg/kg when he is 18 months old.<sup>131</sup> Thereafter, the estimated total requirement of children is 4 to 10 mg/day, the higher figure allowing for establishment of stores. Adolescents require 10 to 20 mg/day.<sup>7</sup>

By far the most common cause of an inade-

quate diet in infancy is excessive dependence on milk.<sup>74,147</sup> Some mothers allow their children to use the bottle as a pacifier and constant companion, and the infants become addicted ("milkoholics"). In one study, inadequate diet was considered to be the only factor in the development of iron deficiency in 20 of 55 infants<sup>74</sup>; it was uncommon in this series to find iron deficiency resulting

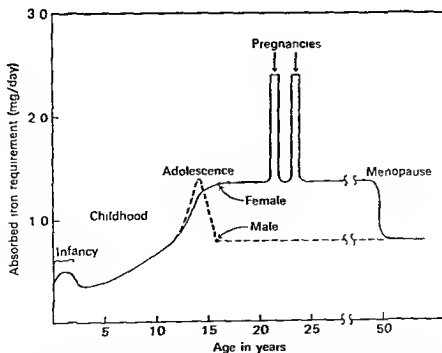


Fig. 17-2. Iron requirements in males and females of various ages. The greatest requirements in relation to food intake occur during infancy. During childhood, requirements are the same in both sexes. The adolescent growth spurt leads to a further increase in iron needs, more so in the male than in the female. Thereafter, with the onset of menstruation, adult female requirements exceed those of the male.

from defective stores at birth unless the diet was also inadequate. "Bakima disease" was attributed to the practice of the Uganda tribe of feeding children almost exclusively on a diet of cows' milk.<sup>97</sup>

### Blood Loss in Infancy

Occult hemorrhage, often without obvious anatomic lesions, has been observed in iron-deficient infants by a number of investigators.<sup>91,112</sup> The process is often accompanied by diffuse disease of the bowel with protein-losing enteropathy and impaired absorption of several nutrients.<sup>81,91</sup> Generalized hypoproteinemia may be observed, along with hypocupremia due to ceruloplasmin loss.<sup>130</sup> Whether this syndrome is a cause or the result of iron deficiency is disputed.<sup>77</sup> In some instances, the abnormalities appear to have been corrected by iron treatment alone.<sup>114</sup> On the other hand, considerable evidence that the disorder results from hypersensitivity to a heat-labile protein in cows' milk has been reported.<sup>112,115</sup> The daily loss of 1 to 4 ml of blood, along with increased serum albumin turnover, was observed while fresh cows' milk was being consumed, and these abnormalities ceased abruptly when heat-treated or soybean-protein feeding formulas were substituted. Furthermore, in infants in whom fresh cows' milk was introduced into the diet at age two months, iron deficiency was considerably more common

than in those receiving prepared formulas during the entire first year of life.<sup>148</sup>

An uncommon cause of blood loss, almost unique to infancy, is bleeding from Meckel's diverticulum.<sup>135</sup> Diagnosis may be difficult prior to abdominal exploration.

### Pregnancy and Lactation

Pregnancy constitutes a major drain on the iron reserves of women of childbearing age. Each pregnancy results in an average loss to the mother of 680 mg of iron (Table 17-6), the equivalent of 1300 ml blood. An additional 450 mg of iron must be available to meet the needs of an expanded blood volume in pregnancy. This does not represent a loss because after delivery the iron is returned to stores, but it must be available during the pregnancy or iron-deficiency anemia will supervene.

Prorated over the full term of pregnancy, the iron requirement amounts to 2.5 mg/day (Table 17-6). Since most of the loss occurs during the third trimester, the requirement is small early in pregnancy and rises to as much as 3 to 7.5 mg/day in the third trimester (Fig. 17-3). These amounts are much greater than can be absorbed from even the best of diets; hence, adequate prenatal care requires iron supplementation. In the absence of supplements, iron-deficiency anemia often occurs, usually becoming apparent in the third trimester.<sup>76</sup>

Table 17-6. Iron Balance in Pregnancy<sup>7</sup>

Iron	Amount (mg)	
	Mean	Range
Lost to fetus	270	(200-370)
Lost in placenta and cord	90	(30-170)
In blood lost at delivery	150	(90-310)
Normal body iron loss	170	(150-200)
Added to expanded red cell mass	450	(200-600)
<b>Total</b>	<b>1130</b>	<b>(670-1650)</b>
Returned to stores when red cell mass contracts after delivery	450	(200-600)
<b>Net Loss</b>	<b>680</b>	<b>(470-1050)</b>
mg/9 mo		
mg/day	2.5	(1.7-4.0)

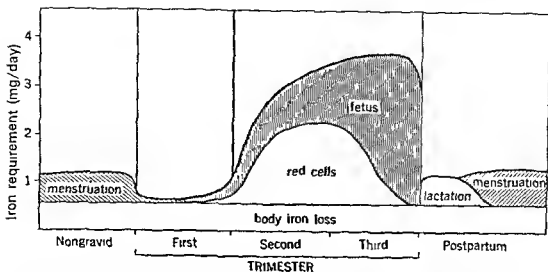


Fig. 17-3. Daily iron requirements in the adult female during pregnancy and postpartum. The requirement includes iron lost from the body and iron for the fetus, for the enlarging red cell mass during pregnancy, and for lactation after birth of the child (From Bothwell and Finch,<sup>61</sup> courtesy of the authors and Little, Brown & Co.)

Lactation results in a daily iron loss of about 0.5 to 1 mg. Since normal menstruation is usually inhibited while breast feeding continues, iron requirements in the lactating woman approximate those of the menstruating woman (Fig. 17-3).

## Clinical Manifestations of Iron Deficiency

Anemia is not a disease; it is a sign of disease. Iron-deficiency anemia is no exception to this rule. In some cases, iron-deficiency anemia is discovered as an incidental finding and the presenting signs and symptoms are related to the disease which led to the deficiency (eg, peptic ulcer). In other cases, manifestations both of the underlying disease and of iron deficiency itself are found. A final group of patients seeks medical attention for symptoms of iron deficiency alone, and the disease process leading to the deficiency is obscure. In the discussion to follow, only the manifestations of iron deficiency will be dealt with. The possible underlying diseases have been discussed in the previous section.

### Age and Sex

In adults, iron deficiency is considerably more common in women than in men (Fig. 17-4), for reasons already discussed. In women, the disorder is seen most frequently during the reproductive years, whereas in men the incidence is relatively high at adolescence, low during young adulthood, and increases thereafter with age (Fig. 17-4).

In infancy, iron deficiency occurs with equal frequency in both sexes. It is usually detected between 6 and 20 months of age<sup>74,181</sup>; the peak incidence occurs at a younger age in infants born prematurely than in those born at term (Fig. 17-5).

### Mode of Presentation

The onset of iron-deficiency anemia is almost invariably insidious and the progression of symptoms is gradual. As a result, patients accommodate remarkably well to advancing anemia and may delay a visit to their physician for prolonged periods: an average of 8 years in the 1930's<sup>27</sup> and in more recent times an average of 3.3 years in women and 1.7 years in men.<sup>60</sup>

Most patients seek medical attention be-

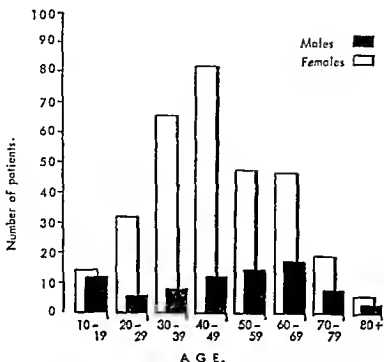


Fig 17-4 Age and sex of 371 adults with iron-deficiency anemia (From Beveridge et al,<sup>60</sup> courtesy of the authors and Oxford University Press)

cause of symptoms of anemia alone. This was true in 63% of 371 patients.<sup>60</sup> Another 16% visited the physician because of symptoms of the disease causing the anemia. In the remaining 21%, anemia was discovered upon evaluation of an unrelated complaint.

#### Fatigue and Other Symptoms of Anemia

Although fatigue is a common complaint of patients who are anemic, it is also common among those who are not. When symptoms in a diseased population are compared with those in a healthy one, mild to moderate iron-deficiency anemia appears to be completely asymptomatic. In the range of 8 to 12 g/dl hemoglobin concentration, there was no correlation between the degree of anemia and the intensity of any of the following symptoms: fatigue, irritability, palpitations, dizziness, breathlessness, or headache.<sup>13</sup> Furthermore, in a controlled study, treatment with iron in amounts sufficient to increase the hemoglobin an average of 2.3 g/dl (from 10.6 to 12.9 g/dl) resulted in no

greater improvement in any of the above symptoms than did treatment with a placebo.

Obviously, at some point, the hemoglobin reaches a level that produces symptoms. A carefully controlled study to determine that level has not been carried out. However, indirect evidence suggests that at least a significant proportion of patients seek medical attention for symptoms of anemia at hemoglobin levels between 7 and 8 g/dl. The average hemoglobin value at first visit in 371 patients was 7.6 g/dl,<sup>60</sup> and 80% of the patients had manifestations that were ascribed to anemia. Patients may develop severe degrees of anemia, with hemoglobin values as low as 4 g/dl, with remarkably few complaints. When signs and symptoms do develop, they usually are manifestations of the cardiovascular adjustments to anemia. These have been discussed in Chapter 13.

In sharp contrast to the conclusion of the studies cited above is the suggestion that even latent iron deficiency, ie, iron deficiency without any anemia at all, may result in fatigue.<sup>3</sup> A group of 44 oonanemic women complaining of fatigue were treated both with

iron and with placebo in random order. Symptomatic improvement on iron was significantly better than on placebo, but only in women whose iron stores were depleted. Others have been unable to confirm this observation.<sup>9,10,13</sup> It seems possible that the patients knew when they received iron because of the well-known change in stool color which accompanies such therapy. If change in color occurred, the placebo effects on fatigue could have been dramatic; in one study, 47% noted improvement in fatigue with placebo treatment alone.<sup>13</sup>

### Epithelial Tissues

Certain abnormalities found in iron-deficient patients are characterized by defective structure or function of epithelial tissues.

Especially affected are the nails, the tongue and mouth, the hypopharynx, and the stomach. These epithelial lesions tend to occur together in the same patients at the same time,<sup>60</sup> but may also occur as isolated findings. Less well established are effects on the skin, hair, and nose.

The incidence of epithelial abnormalities in a large group of iron-deficient, anemic patients studied in England between 1941 and 1960 is given in Table 17-7.<sup>60</sup> These figures (the most recent available) do not differ greatly from those found in 1933 in a review of 260 patients from several parts of the world, including the United States.<sup>27</sup> It has been claimed<sup>34</sup> that the epithelial manifestations of iron deficiency now are uncommon in the United States. We tend to agree, but there is no good statistical evidence to

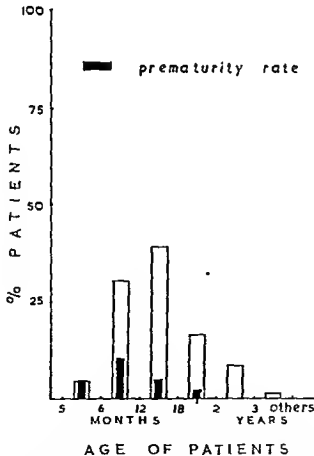


Fig. 17-5 Age distribution of 533 infants with iron-deficiency anemia. The illness occurs at a younger age in prematures than in full term infants (From Lovnic et al.,<sup>131</sup> courtesy of the authors and Australasian Medical Publishing Co.)

**Table 17-7. Incidence of Epithelial Lesions in Iron Deficiency**

Organ	Finding	Incidence <sup>a</sup>
Nails	Flattening	10%
	Koilonychia	18%
	Total abnormal	28%
Tongue	Sore tongue	11%
	Absence of filiform papillae	11%
	Lesser degrees of papillary atrophy	28%
	Total abnormal	39%
Mouth	Angular stomatitis	14%
Hypopharynx	Dysphagia	7%
Stomach	Achlorhydria	41%
	Gastritis†	74%
Nose	Ozena	0

<sup>a</sup>In 371 adult British patients seen from 1941-1960.<sup>60</sup>

†By gastric biopsy. The figures include superficial gastritis, atrophic gastritis and gastric atrophy. In controls, gastritis was found in 29% of biopsies.<sup>149</sup> Similar results have been reported by others.<sup>174</sup>

either support or refute the opinion. It is also of interest to note that the incidence of dysphagia is said to be very low in areas of East and Central Africa where iron deficiency is almost universal.<sup>177</sup>

### Nails

The fingernails may become brittle, fragile, or longitudinally ridged, but such findings are quite nonspecific. Alterations more typical of iron deficiency are thinning, flattening, and, finally, the development of *koilonychia*, concave or "spoon-shaped" nails (Fig. 17-6). *Koilonychia* has been thought to be rare in iron-deficient infants,<sup>183</sup> but out of 400 babies attending a well-baby clinic in West Virginia, 22 (5.5%) were found to have *koilonychia*, and at least 18 of these appeared to be iron deficient<sup>172</sup> (Fig. 17-7).

*Koilonychia*, while strongly suggestive of iron deficiency,<sup>144</sup> can develop with long repeated exposure to hot soapsuds and other caustic agents.<sup>162</sup> When *koilonychia* is associated with a variety of skin conditions, such

as fungal disease, the nails are likely to be irregularly pitted. ✓

### Tongue and Mouth

Atrophy of the lingual papillae, the most common of the epithelial changes (Table 17-7), may be accompanied by soreness or burning of the tongue, occurring either spontaneously or stimulated by food or drink, and by varying degrees of redness.<sup>60,141,176</sup> The filiform papillae over the anterior two thirds of the tongue are the first to atrophy, and may disappear completely. In severe cases, fungiform papillae also may be affected, leaving the tongue completely smooth and waxy or glistening in appearance<sup>176</sup> (Fig. 17-8). A biopsy study confirmed the absence of filiform papillae in a proportion of iron-deficient patients and also demonstrated loss of keratohyalin granules in lingual epithelial cells.<sup>183</sup> These changes were reversed after one to two weeks of iron therapy.

Angular stomatitis is characterized by ulcerations or fissures at the corners of the mouth. In addition to iron deficiency, edentia, poorly fitting dentures, and other nutritional deficiencies contribute to the development of such lesions.<sup>60</sup>

### Dysphagia

The association of dysphagia, angular stomatitis, and lingual abnormalities with hypochromic anemia in middle-aged women was first described independently by Paterson<sup>185</sup> and by Kelly<sup>179</sup> in 1919. Vinson, in 1922, reported 69 similar cases and attributed the first description of the syndrome to apparently unpublished observations of Plummer.<sup>189</sup>

Patients with sideropenic dysphagia<sup>190</sup> (Paterson-Kelly syndrome,<sup>177</sup> Plummer-Vinson syndrome) note the usually gradual onset of dysphagia; they describe the site of the discomfort as being localized sharply to the area of the neck near the cricoid cartilage. They experience difficulty in swallowing solid foods and have little problem with liquids. If the patient is untreated the dysphagia





Fig 17-6 Fingernails of an iron-deficient adult (below) compared with those of a normal subject (From Rosenbaum and Leonard,<sup>127</sup> courtesy of the authors and *Annals of Internal Medicine*)

grows worse, and ultimately his diet becomes so restricted as to interfere with nutrition.

The most common anatomic lesion is a "web" of mucosa at the juncture between the hypopharynx and esophagus. These webs, which may be multiple, usually extend from the anterior wall into the lumen of the esophagus, but they may encircle the lumen completely, forming a cuff-like structure. In other patients a benign stricture, with or without a web, may be found; and the opening into the esophagus at the cricoid area may be reduced to the size of a pinhole or slit.<sup>144</sup>

Both webs and strictures can often be demonstrated by x rays of the lateral aspect

of the neck taken after barium swallow<sup>173,190</sup> (Fig. 17-9). Multiple exposures or cineradiography are required for optimal demonstration of these abnormalities. Radiologic findings in this syndrome in 55 Welsh patients, of which 53 were women, were as follows: 38 (69%) with webs, including five with two webs; six (11%) with benign stricture of the esophagus; five (9%) with carcinoma, presumably a late complication since all had had dysphagia for over 20 years; and seven (13%) with no demonstrable lesion.<sup>177</sup>

On biopsy, the webs appear to be constructed of normal epithelium with underlying loose connective tissue, in which there



Fig 17-7. Koilonychia in a 1½-year-old child with iron-deficiency anemia

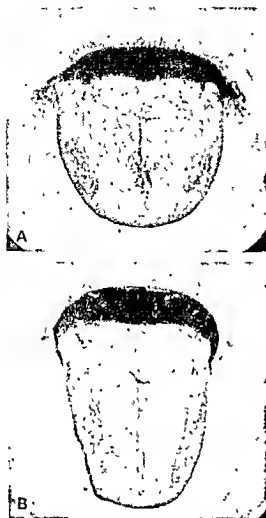


Fig 17-8 Tongue of a patient with iron-deficiency anemia showing moderately severe papillary atrophy before therapy (A) and restoration after iron repletion (B) (Courtesy of RW Monto, Detroit Michigan)

may be a chronic inflammatory reaction.<sup>163,170</sup> In a small proportion, hyperchromatic nuclei and increased mitotic activity may be observed in the basal cell layer. Biopsy of the stricture demonstrates chronic, nonspecific inflammation and degeneration of striated muscle. Carcinoma in situ was found in 5% of biopsy studies.<sup>170</sup> Carcinoma in the postcricoid area as a late complication of the syndrome was described in the original reports of Paterson and Kelly.<sup>179,185</sup>

For relief of the dysphagia, it is usually necessary to rupture the webs and/or dilate the stenosis by means of bouginage. Reple-

tion of the iron stores will not, by itself, suffice.<sup>141</sup>

### *Stomach*

Gastric biopsy demonstrates the presence of "gastritis" in about 75% of patients with iron-deficiency anemia as compared to about 29% in controls.<sup>169,174</sup> Depending on the criteria used by various authors, the gastritis has been classified as "superficial," "mildly" to "severely" atrophic, or as "gastric atrophy." Such lesions are nonspecific and may be indistinguishable from those seen in association with pernicious anemia or occasionally in apparently normal, older adults (Chapter 15, page 606).<sup>182</sup> Most investigators have concluded that chronic gastritis of this sort produces few, if any, symptoms.

Associated with the gastritis are varying degrees of reduction in gastric secretion. With progression of gastric damage, there is loss of ability to secrete acid, pepsin, and intrinsic factor, in that order.<sup>144</sup> The proportion of iron-deficient patients found to have achlorhydria depends on the methods used to stimulate secretion. Thus, in early studies, an incidence as great as 80% was observed.<sup>28</sup> The data in Table 17-7 came largely from the use of single-dose histamine or Diágnex Blue testing. Of 70 patients subjected to the more stringent augmented histamine test, achlorhydria was found in only 16% and extreme hypochlorhydria in 7%. With this technique,<sup>144</sup> achlorhydria is most unusual in unselected hospital controls.

Of the same 70 patients, in 6% intrinsic factor secretion was impaired or absent. In another series, defective vitamin B<sub>12</sub> absorption was found in 7 of 22 patients with iron deficiency complicated by histamine-fast achlorhydria.<sup>168</sup> These reports probably indicate the severity of the gastric damage in iron deficiency, rather than coincidental pernicious anemia.

Antibodies to gastric parietal cells (Chapter 15, page 606) are found in about one third of patients with iron deficiency.<sup>178,191</sup> Although the mechanism of production of these antibodies is not understood, they are more

likely to be a manifestation of gastritis than the cause.<sup>143</sup>

### *Ozena*

Ozena is a disease of unknown cause characterized by chronic atrophy of the nasal mucosa associated with a foul-smelling discharge. Although uncommon in the United States, ozena occurs relatively frequently in southeastern Europe. There, an association between ozena and iron deficiency was observed.<sup>165</sup> About three fourths of 136 patients with ozena had hypochromic anemia or hypoferrremia or both. Furthermore, improvement or complete relief was observed following iron therapy. On the basis of these observations as well as epidemiologic information,

it was concluded that ozena is a manifestation of chronic iron deficiency. On the other hand, ozena is rare in Norway even though iron deficiency is common.<sup>164</sup> There, only nine of 24,176 patients visiting an ear, nose, and throat clinic were found to have ozena. None of the nine had anemia, hypochromia, or hypoferrremia, and eight had adequate iron stores in the bone marrow. Others have reported failure of iron therapy in ozena.<sup>184</sup> It therefore appears that if iron deficiency plays any role at all in the cause of ozena, it is but one factor in a multifactorial disease.

### *Pica*

Pica has been previously alluded to as a possible cause of iron deficiency (page 641).



Fig. 17-9. Esophageal web (arrow) in Plummer-Vinson syndrome

It may also be a striking manifestation of the disease. Hippocrates wrote that a "craving to eat earth" was associated with "corruption of the blood."<sup>1</sup> "Perversion of the appetite" was a prominent symptom in chlorosis.<sup>23</sup>

Pagophagia, defined as the purposeful eating of at least one tray of ice daily for two months, was reported in 25 adult patients with iron deficiency.<sup>60</sup> The ingestion of ice averaged nearly 2 kg per day, and some patients ate an astounding 4 to 9 kg per day. This dramatic symptom was relieved within 1 to 14 days after iron was administered. Another study found that pagophagia was a symptom of iron deficiency in 23 of 38 consecutive adult patients; iron therapy was curative.<sup>186</sup>

Other forms of pica occur particularly in children and may also respond to iron therapy.<sup>103</sup>

### The Spleen

The spleen is enlarged in about 10% of patients.<sup>27,60</sup> The degree of enlargement is slight; usually only the tip is felt. There are no specific pathologic changes in the organ, and the pathogenesis of the enlargement is unknown.

### Genitourinary System

Disturbances in menstruation are frequent, but it is sometimes difficult to be certain whether they are the cause or the result of deficiency. Several investigators have reported that menorrhagia was relieved by iron therapy.<sup>27,171,188</sup> However, in a study in which <sup>51</sup>Cr-labeling was used to measure menstrual blood loss, the flow did not decrease and often even became heavier after therapy.<sup>175</sup>

### Neuromuscular System

There may be neuralgic pains, vasomotor disturbances, or numbness and tingling. The last-mentioned complaints have been observed in 15 to 30% of patients with chronic hypochromic anemia.<sup>27</sup> More serious symp-

oms, such as disturbances of gait and objective neurologic findings, are unusual.<sup>187</sup>

Rarely, iron deficiency may lead to increased intracranial pressure, papilledema, and the clinical picture of pseudotumor cerebri.<sup>167</sup> About 44 such cases have been reported, and in 29 of these the information provided was adequate to exclude occult intracranial lesions and to establish the fact that the condition was corrected after iron therapy. The pathogenesis is unknown, but it was suggested that severe anemia and reduced tissue iron enzymes might lead to cerebral anoxia and edema, as occurs in carbon monoxide intoxication.<sup>167</sup>

### Skeletal System

In children with iron-deficiency anemia of long standing, there may be changes in the skull similar to those found in association with thalassemia or chronic hemolytic anemia.<sup>161,168,180</sup> The diploic spaces may be widened and the outer tables thinned, at times with vertical striations producing a hair-on-end appearance. In addition, there may be abnormalities of the long bones, especially the metacarpals and phalanges, with expansion of the medulla and thinning of the cortices.<sup>180</sup> The changes are thought to result from expansion of the erythroid marrow during the period of bone growth and development.

## Laboratory Findings

### The Blood

#### Erythrocytes

The degree of anemia depends on the presenting circumstances. If discovered when the patient is evaluated for an underlying or unrelated disease, the anemia may be mild. If symptoms of anemia are the presenting complaint, the blood hemoglobin is usually 8 g/dl or lower. In 371 English patients, the mean hemoglobin was 7.6 g/dl, with values of less than 4 g/dl in eight, and greater than

11 g/dl in five.<sup>60</sup> In 115 patients in the United States the VPRC ranged from 0.10 to 0.40 l/l, with a mean of 0.30 l/l (hemoglobin 8.4 g/dl).<sup>2</sup> A similar range of values has been reported by others, in both adults<sup>219</sup> and children.<sup>220</sup> Because of the hypochromia, the hemoglobin is usually reduced to a greater degree than the VPRC.

The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) are all reduced in the usual patient. The average MCV is 74 fl (range, 53 to 93), MCHC 28 g/dl (22 to 31), and MCH 20 pg (14 to 29).<sup>2,219</sup> Similar values were found in infants.<sup>220</sup> The degree of change in the red cell indices is related partly to the duration<sup>8</sup> and partly to the severity of the anemia. In mild iron deficiency of short duration the indices may be normal.<sup>204</sup>

Both the percentage and the absolute number of reticulocytes tend to be normal or slightly increased,<sup>2,219</sup> but, rarely, they may be reduced. In experimentally induced iron deficiency in certain animal species, including rats and pigs, a pronounced reticulocytosis is seen.<sup>207,219</sup>

The chief finding on blood smear is the

poverty of hemoglobin in the individual red corpuscles, as indicated by an exaggeration of their central pallor (Fig. 17-10). The more severe the anemia, the greater the degree of this change and the more numerous the corpuscles affected. In extreme grades of hypochromic anemia, most of the red corpuscles are mere rings. Tiny microcytes and a moderate number of poikilocytes, particularly tailed and elongated elliptical forms, also are found. In almost all instances, however, a variable number of well-filled red corpuscles are present and some macrocytes, often polychromatophilic, can be distinguished. These may represent cells made during occasional periods of increased iron availability, for example, after a meal rich in iron. The number of such cells is increased as iron is made available. The diagnosis of iron-deficiency anemia from blood smear alone may be difficult.<sup>212</sup>

The fragility of the red corpuscles may be within the normal range, but often there is increased resistance to destruction in hypotonic salt solutions, a pale layer of unbroken cells remaining in tubes of salt concentration as low as 0.21 g/dl. Extreme grades of corpuscular resistance, however, such as are

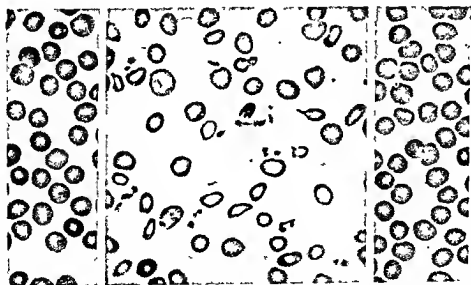


Fig. 17-10. Blood smear from a patient with hypochromic, microcytic anemia due to iron deficiency. Red corpuscles from normal blood are shown, for comparison, on each side (Wright's stain,  $\times 900$ )

found in thalassemia (Chapter 26) are unusual. The abnormal osmotic fragility returns to normal following adequate therapy.<sup>221</sup>

### *Leukocytes and Platelets*

The leukocytes are usually normal in number, but there may be slight absolute granulocytopenia in long-standing cases.<sup>225</sup> A few hypersegmented neutrophils may be found,<sup>203</sup> but it is sometimes difficult to exclude the possibility that folate deficiency in the recent past is responsible for the finding.<sup>216</sup> A fresh hemorrhage of large volume may cause slight neutrophilic leukocytosis, with even the appearance of an occasional myelocyte. In hookworm anemia, eosinophilia is common.<sup>121</sup>

The platelet count usually is increased to about twice the normal level, and values return to normal after therapy.<sup>215,223</sup> It has been claimed that this thrombocytosis reflects continuing blood loss rather than iron deficiency.<sup>210</sup> However, dietary iron deficiency in rats also is accompanied by thrombocytosis.<sup>223</sup> In some patients with iron-deficiency anemia that is severe or of long standing, mild thrombocytopenia may be observed, possibly because of complicating factors such as folate deficiency or splenic sequestration.<sup>210,215</sup>

### *Bone Marrow*

The bone marrow is characterized by erythroid hyperplasia of a variable, but generally mild to moderate, degree. Of the nucleated cells in marrow aspirates from iron-deficient patients,  $25 \pm 9.8\%$  were erythroblasts as compared with  $16.9 \pm 2.7\%$  in normal subjects.<sup>206</sup> Furthermore, cellularity of the marrow, as judged from sections of marrow fragments, was moderately increased in the deficient patient. There was no good correlation between any of these changes and the degree of anemia. Striking nuclear distortions, resembling those found in dyserythropoietic anemias (Chap. 19), may sometimes be observed in the marrow.<sup>216a</sup> Karyorrhexis and nuclear budding are particularly common, but

multinuclearity, nuclear fragmentation, and even intranuclear bridging may be observed. When therapy is given, erythroid hyperplasia initially increases, but as the blood is restored to normal the cellularity of the marrow likewise becomes normal.

The individual normoblasts appear small, and may have scanty cytoplasm, often with irregular, ragged borders. Findings in the marrow after staining for iron are described below.

### *Kinetic Studies*

Studies employing the  $DF^{52}P$  or Ashby methods demonstrate that the red cell survival is somewhat shortened<sup>209,217</sup> (Table 17-8). Only when the change is severe can it be detected with the  $^{51}Cr$  method.<sup>209,218</sup> That the shortened survival results from an intracorporeal defect has been demonstrated by cross-transfusion studies.<sup>209,217</sup> Furthermore there is excellent correlation between the degree to which red cell survival is shortened and the proportion of morphologically abnormal cells on blood smear.<sup>209</sup> The principal site of destruction appears to be the spleen.<sup>218</sup> One study indicated that administration of prednisone prolongs red cell survival in iron deficiency, whereas the effect of iron on survival is delayed until the abnormal cells disappear.<sup>211</sup>

Ferroketic studies have been puzzling because they seem to show that, despite a low plasma iron, iron clearance is so rapid that a normal amount of iron is delivered to marrow, where it is utilized more efficiently than normal, resulting in an apparent net increase in hemoglobin production as represented by the erythrocyte iron turnover rate (EIT) (Table 17-8). These data are clarified by the observation that there was a significant component of "ineffective erythropoiesis" in iron-deficient rats, as demonstrated by marked augmentation of the "early-labeled" bilirubin fraction (Chapter 5).<sup>222</sup> This would suggest that a proportion of cells in iron deficiency are so defective that they are rapidly destroyed, and that the iron they contain is quickly reutilized. The iron traveling

**Table 17-8. Erythrokinetic and Ferrokinetic Measurements in Iron Deficiency<sup>209,213,217</sup>**

<i>Measurement</i>	<i>Normal†</i>	<i>Iron Deficient†</i>
Red cell survival, Ashby (days)	117	73
Red cell survival, DF <sup>32</sup> P (days)	117.5	78, 54*
Red cell survival, <sup>51</sup> Cr (t <sub>1/2</sub> , days)	30	26, 18*
<i>Ferrokinetics</i>		
Plasma iron µg/dl	105	21
Iron clearance (t <sub>1/2</sub> , minutes)	86	20
Plasma iron transport (mg/day/dl)	0.7	0.8
Red cell iron utilization (%)	80	93
Erythrocyte iron turnover (mg/day/dl)	0.56	0.74

\*The lower figures are from nine subjects with hookworm disease in whom the anemia was unusually severe and prolonged

† Values are means

through such a shunt pathway would account for the ferrokinetic peculiarities.

### Iron Metabolism

In sera of adults with iron-deficiency anemia, iron concentration is reduced to an average of 28 µg/dl (range 10 to 61).<sup>2</sup> The total iron-binding capacity (TIBC) often is increased, but it may be normal or even decreased (Fig. 16-3). In one study, the average value was 346 µg/dl, range 170 to 460.<sup>2</sup> The patients with reduced TIBC values tended to have hypoalbuminemia as well. Saturation of transferrin is always reduced to less than 16% and averages 7%. Values for serum iron, TIBC, and transferrin saturation are similar in iron-deficient children, except that the increase in TIBC is more consistent.<sup>220</sup>

Reticuloendothelial iron stores are absent or severely reduced in marrow or liver. Less than 10% (average 2.5%) of the marrow normoblasts are sideroblasts.<sup>2</sup>

Comparison of these abnormalities with those in other hypochromic anemias is discussed in Chapter 16.

### Iron Enzymes

In addition to being a component of hemoglobin, iron forms a part of a number of important tissue enzymes, including cytochrome c, cytochrome oxidase, catalase,

myoglobin, succinic dehydrogenase, aconitase, and possibly glutamate formiminotransferase.<sup>203,205,215,224</sup> In experimentally induced iron deficiency in animals, the activity of many of these enzymes becomes reduced and can be restored by iron treatment. Various tissues, including brain, heart, liver, kidney, and muscle, may be affected.

Studies in man obviously are more difficult than in experimental animals because tissues are not so readily available. However, it has been demonstrated that catalase is decreased in erythrocytes of human subjects with iron deficiency.<sup>202</sup> Also, cytochrome oxidase activity is reduced in buccal epithelium<sup>9</sup> and in intestinal epithelium.<sup>203</sup> In the latter, when iron is given, regeneration of cytochrome oxidase begins in newly formed cells at the base of the intestinal villus, and over a three- to four-day period these cells gradually replace the older, deficient cells.<sup>203</sup>

The pathophysiologic importance of changes in tissue enzymes remains uncertain. It has been suggested that the abnormalities may partially account for the symptom of fatigue.<sup>3</sup> The significance of fatigue as a symptom of iron deficiency was discussed earlier (page 650). Another suggestion is that the epithelial manifestations of iron deficiency are related to impaired activities of these enzymes. However, no correlation could be found between buccal cytochrome oxidase activity and atrophic mucosa.<sup>9</sup>

## Lactate Acidosis

Lactate acidosis was observed in a single patient with very severe iron-deficiency anemia (blood hemoglobin 1.5 g/dl).<sup>214</sup>

## Management

### Measures Directed at the Cause of the Anemia

Every effort must be made to define the etiologic factor. This should be possible in about 80 to 85% of patients.<sup>60</sup> In the remainder, it is possible that the underlying disease is in remission; therefore, continued observation for new clues as to its nature is warranted. Once the etiologic diagnosis is made, appropriate treatment becomes possible. Therapy may be as simple as discontinuing salicylate ingestion or it may require complex decisions between medical and surgical approaches to bleeding gastrointestinal lesions. It is in the area of primary diagnosis and therapy that the difficult and challenging problems in management of iron deficiency lie. By comparison, repletion of total body iron stores is relatively simple.

### Measures Designed to Replenish Total Body Iron

Iron is highly effective in treating iron deficiency. *It has no other legitimate therapeutic use*, in particular it exerts no effect in any of the numerous anemias which are not due to iron deficiency. The practice of giving

iron indiscriminately in undiagnosed anemia cannot be deplored too emphatically. It will certainly do no good, may do harm, and may delay institution of other, more suitable, therapeutic measures.

Iron can be administered orally, intramuscularly, or intravenously. Of these, the oral route is by far the safest and least expensive. Iron-deficient patients who cannot tolerate oral therapy or who fail to respond to it when properly given are most unusual.

### Oral Iron Therapy

**RECOMMENDED PREPARATIONS AND ADMINISTRATION.** The standard preparation for oral use is ferrous sulfate. It is effective, well-tolerated, and inexpensive. If equivalent amounts of elemental iron are given, two other iron salts are equally satisfactory, have about the same incidence of side effects, and cost only slightly more than ferrous sulfate. These are ferrous gluconate and ferrous fumarate (Table 17-9).

The dose is best calculated in terms of elemental iron. For adults, the optimal response occurs when about 200 mg of elemental iron are given each day. Three ferrous sulfate tablets or 5 teaspoons of ferrous sulfate elixir provide this daily dose. To achieve similar doses with ferrous gluconate, 5 tablets per day are required. Ferrous fumarate can be given two or three times a day, depending on the tablet size (Table 17-9).

Iron is absorbed more completely when the stomach is empty; when taken after or with

Table 17-9. Standard Therapeutic Oral Iron Preparations

Preparation	Size	Iron Content	Usual Adult Daily Dose	Approximate Cost per Month
<b>Ferrous sulfate</b>				
Tablets (hydrated)	300 mg	60 mg	3 tablets	\$1.50
Tablets (exsiccated)	200 mg	60 mg	3 tablets	\$1.50
Syrup and elixirs	40 mg/ml	8 mg/ml	25 ml (5 tsp)	\$4.00
Pediatric drops	125 mg/ml	25 mg/ml	—	—
<b>Ferrous gluconate</b>				
	300 mg	37 mg	5 tablets	\$3.00
<b>Ferrous fumarate</b>				
	200 mg	67 mg	3 tablets	\$2.50
	300 mg	100 mg	2 tablets	



a meal, absorption was found to decrease by 40 to 50%.<sup>235</sup> However, gastrointestinal irritation is common when the stomach is empty; consequently it is usually better to advise that the tablet be taken immediately after or even with a meal; the gain in patient acceptance may be more important than the reduced absorption of iron. The daily dose, furthermore, should be divided roughly in accordance with the size of the meal. Even two tablets may be readily tolerated after a large meal, whereas one may cause discomfort if the stomach is empty.

As Blaud recognized more than a century ago, *tolerance for iron salts usually is improved by giving a small dose at first and increasing the amount in the course of several days to the full dose of 3 tablets. Without such a gradual increase in dosage, gastrointestinal side effects are more common.*

For children, 1.5 to 2.0 mg elemental iron per kg body weight three times a day are effective. Palatable elixirs and syrups are the most satisfactory pediatric preparations. Children usually tolerate this form of therapy on an empty stomach.

Studies of the absorption of medicinal iron in deficient subjects are difficult because many patients continue to bleed and therefore lose iron. One study circumvented this problem by using eight healthy male volunteers in whom iron-deficiency anemia (Hb 9.2 to 11.4, MCHC 28.3 to 31.7) was induced by phlebotomy.<sup>244</sup> At therapeutic doses of ferrous sulfate (222 mg elemental iron per day), 13.5% (range 10.6 to 18.7%) was absorbed during the first 20 days of therapy. It was calculated that 605 mg of iron were absorbed during this period. As hemoglobin regeneration occurred, absorption decreased; in the period 21 to 30 days after therapy was begun, the average value was 5.1% (2.3 to 6.0). Since the number of individuals studied was small, these figures can serve as only a rough guide to expected absorption in others. Individual variations are great and absorption may be vastly different at different degrees of anemia and in the presence of complicating illnesses.

The 200-mg daily dose will produce a maximum rate of hemoglobin regeneration.

It is not necessarily true, however, that the maximum rate is the only legitimate therapeutic goal. If the underlying disease has been corrected and if the anemia is mild to moderate, a slower rate of response may be acceptable. Thus, if such patients cannot tolerate 200 mg a day of elemental iron as ferrous sulfate, it is reasonable to reduce the dose to about 100 mg a day. A convenient way to accomplish this is with ferrous gluconate, since 3 tablets a day will provide 110 mg iron. Because less iron is given, tolerance is improved. In this circumstance, speed of response is exchanged for patient acceptance. Therapy will probably be prolonged, but this may be no great disadvantage to the selected patients.

Regardless of the form of oral therapy used, it is important to continue treatment for at least six months after the anemia has been relieved. If this is not done, relapse is common. The continued therapy allows for repletion of iron stores.

**SIDE EFFECTS.** Some patients given iron by mouth complain of gastrointestinal symptoms, including heartburn, nausea, abdominal cramps, and diarrhea. In many instances, both the frequency and severity of the symptoms have been greatly exaggerated. Functional gastrointestinal symptoms are common in the absence of medication; furthermore, they are greatly influenced by suggestion.

Many studies of gastrointestinal intolerance to iron have not included adequate controls. In one excellent, double blind study, ferrous sulfate, ferrous gluconate, ferrous fumarate, and placebo were administered in identically appearing tablets.<sup>243</sup> The elemental iron dose was 222 mg per day. The incidence of gastrointestinal side effects was 13% in subjects taking placebo tablets and 25% in those taking iron. No significant differences were found among the three iron salts. Thus, about 12% had symptoms that could reasonably be ascribed to iron.

It is probable that side effects are related to dose. In another controlled study, iron was given in a dose of 105 mg/day.<sup>247</sup> About 20% of the members of both the iron and the

placebo groups complained of side effects. Thus, at this dose level there were no side effects which could be attributed to iron. In an uncontrolled study limited to young men, 150 mg elemental iron were given as ferrous sulfate per day. No gastrointestinal symptoms were experienced.<sup>236</sup> These studies suggest that patients intolerant of full therapeutic doses may be able to take smaller ones, especially if appropriate psychologic tactics are employed.

**OTHER PREPARATIONS.** A great variety of compounds containing iron have been promoted from time to time with claims of greater effectiveness, greater tolerance, and less toxicity than ferrous sulfate, ferrous gluconate, or ferrous fumarate. In general, these compounds are considerably more expensive than ferrous sulfate. Often, allegations of significant therapeutic superiority or reduced toxicity have not been substantiated.<sup>34,238</sup>

If given in sufficient quantities both ascorbic acid and succinic acid increase absorption of ferrous iron. In the case of ascorbate, at least 200 mg are necessary for each 30 mg of iron, and the increase in absorption at that level is about 30%.<sup>235</sup> However, ascorbate also increases the side effects of iron therapy<sup>213</sup>; thus, it appears that an increase in the dose of iron would achieve the same result at lower cost. Furthermore, many of the available iron and ascorbate preparations contain only trifling amounts of ascorbate.

Unlike ascorbic acid, *succinic acid* promotes iron absorption without increasing the side effects.<sup>243,244</sup> Again, relatively large amounts are required approximately 185 mg succinic acid per 37 mg of iron. In this ratio and with an elemental iron dose of 222 mg per day, the observed absorption in iron deficiency was 21% during the first 20 days of treatment as compared to 12.5% without succinate.<sup>244</sup> It was calculated that the total amount of iron absorbed in 20 days was 940 mg with succinate and 608 without. Iron-succinic acid preparations are not available in the United States, but ferrous succinate is in use in Great Britain. There, the cost is about eight times that of ferrous sulfate.<sup>144</sup> In most

cases, it is doubtful that the increment in absorption justifies the extra cost.

Enteric coated preparations are designed to reduce side effects by retarding dissolution of the iron. However, this effect may markedly decrease absorption, especially in achlorhydric individuals, whose gastric juice cannot dissolve the coating.<sup>31</sup> Sustained release preparations also reduce side effects by retarding dissolution, but in so doing the most actively absorbing regions of the intestine are bypassed; hence, absorption is reduced.<sup>234,237,251</sup> Certain iron compounds are poorly soluble and poorly absorbed, including ferric salts, ferrous carbonate, and ferrous sulfate when mixed with aluminum and magnesium hydroxides. Addition of copper, cobalt, molybdenum, and a variety of other minerals as well as vitamins and "hematinics" confers no therapeutic advantage and adds considerable expense.

**FAILURE OF ORAL IRON THERAPY.** It is not uncommon to encounter patients who are said to have iron-deficiency anemia that is unresponsive to oral therapy. Often such individuals are referred to the hematologist with the suggestion that iron absorption is defective, but that hypothesis usually turns out to be incorrect. In approximate order of importance, the following possible explanations for failure to respond to iron given orally should be considered: (1) incorrect diagnosis; (2) complicating illness; (3) failure of patient to take prescribed medication; (4) inadequate prescription (dose or form); (5) continuing iron loss in excess of intake; and (6) malabsorption of iron.

Of paramount importance is a review of the data upon which the diagnosis of iron deficiency anemia was based along with judicious repetition of any laboratory procedures that might have yielded erroneous information. At times, even though iron deficiency is present, a coexisting cause for anemia may impair response. Common examples are iron deficiency as a complication of the anemia of chronic disorders in rheumatoid arthritis, and so-called "dimorphic anemia" in which iron deficiency and pernicious anemia coexist.

Even apparently intelligent and cooperative patients may fail to take medications as prescribed.<sup>250</sup> As a check on this possibility, it is sometimes wise to ask the patient to bring the medication bottle to the physician for a "pill count." As has been mentioned, some available iron preparations contain inadequate amounts of iron, or are poorly absorbed because of such factors as enteric coatings or reduced solubility. In these instances, simply prescribing ferrous sulfate can bring about response.<sup>251</sup> In occasional patients, blood loss is so great that oral iron therapy cannot keep up with it. If this situation is not correctable, as may be the case, for example, in hereditary hemorrhagic telangiectasia, parenteral therapy must be given. Finally, a rare patient may be unable to absorb iron, although even patients with sprue or total gastrectomy usually are able to absorb adequate amounts of ferrous sulfate.

### Parenteral Iron Therapy

Parenteral iron therapy is as effective but somewhat more dangerous and considerably more expensive than oral therapy. Nevertheless, there are certain clinical situations in which failure of oral therapy is to be expected. It should be emphasized that a history of failure to respond to oral iron is not, by itself, an indication for parenteral therapy. The reasons for failure must be analyzed as outlined above.

Parenteral iron therapy is indicated when the patient (1) is unable to tolerate iron compounds given orally; (2) repeatedly fails to heed instructions or is incapable of accepting or following them; (3) loses iron (blood) at a rate too rapid to be compensated for by the oral intake (eg, hereditary hemorrhagic telangiectasia); (4) has a disorder of the gastrointestinal tract, such as ulcerative colitis, in which symptoms may be aggravated by iron therapy; or (5) is unable to absorb iron from the gastrointestinal tract.

**PREPARATIONS AND ADMINISTRATION.** Available preparations include iron-dextran complex (Imferon) and iron sorbitex (Jecto-

fer), both of which contain 50 mg of iron per ml of solution. The total dose of parenteral agents may be calculated from the amount of iron needed to restore the hemoglobin deficit plus an additional amount to replenish stores. One formula that allows for both is as follows<sup>144</sup>:

$$\begin{aligned} \text{iron to be injected (mg)} \\ = (15 - \text{patient's Hb}) \times \text{body weight} \times 3 \\ \quad \quad \quad \text{(g/dl)} \quad \quad \quad \text{(kg)} \end{aligned}$$

The most extensively used preparation is iron-dextran complex which can be given intramuscularly or intravenously. The intramuscular injections should always be made deep into the upper outer quadrant of the buttock, and the skin should be laterally displaced prior to injection (Z-track technique) to prevent staining of the skin. A test dose of 0.5 ml should be given initially to test for hypersensitivity. Thereafter, usually no more than 2.5 ml are given per injection, but both buttocks may be injected daily for a total of 5 ml (250 mg) per day. Approximately 65% is absorbed from the injection site in 72 hours.<sup>252</sup> An average of about 25% (range 11 to 52%) remains at the injection site for at least four weeks and may be essentially unavailable.<sup>256</sup>

Iron-dextran complex can also be given intravenously. The special advantage of this route is that larger doses can be given in a single injection and the discomfort and inconvenience of repeated intramuscular injections thereby avoided. Since the intravenous route may be more dangerous, however, its use should be reserved for special situations in which rapid replenishment or repeated large doses are needed, as in continuous blood loss. Two methods have been used.<sup>254</sup> After testing for hypersensitivity, 5 to 10 ml of undiluted iron-dextran complex may be administered over a period of five minutes. Alternatively, the entire dose, as calculated by the formula given above, may be given diluted in a ratio of 5 ml iron-dextran complex to 100 ml saline solution. This method, sometimes called total dose infusion or TDI, has not yet gained approval of the U.S. Food and Drug Administration,

but has been used extensively in Great Britain and continental Europe. Glucose solutions may also be used as the diluent, but the incidence of phlebitis is greater than when saline solution is employed. The initial flow rate should be 20 drops/min for five minutes; then, if no side effects are observed, the rate may be increased to 40 to 60 drops/min.

*Iron sorbitex* (iron sorbitol-citric acid complex, Jectofer)<sup>239 245</sup> is a low molecular-weight (5000) preparation for intramuscular use. It is much more rapidly absorbed from the injection site than is iron-dextran complex; less than 15% remains after 24 hours. About 25% is excreted into the urine, and the calculated total dose should take this loss into account. The dose should not exceed 1.5 mg/kg or a total of 100 mg per day.

**SIDE EFFECTS.** Staining of the skin may follow intramuscular injection of iron-dextran complex. There may be pain at the injection site, approximately equivalent to that produced by a penicillin injection. Regional lymph nodes may become tender for several weeks. Phlebitis occurs after intravenous administration in as many as 25% of the injections when dextrose solution is used as diluent, but is less frequent when saline solution is used. With both the intravenous and intramuscular routes, various systemic reactions occur, including headache, flushing, febrile reactions, arthritis, urticaria, nausea, and bronchospasm. However, the overall incidence of such side effects (excluding fever and arthralgia) in 2400 patients given iron-dextran complex intravenously was only 1 to 2%.<sup>254</sup> Furthermore, most reactions were mild and transient. However, anaphylaxis has been observed following both intramuscular and intravenous injections and may be fatal.<sup>232</sup> The clinician should be prepared for the possibility of anaphylaxis and have epinephrine, oxygen, and facilities for resuscitation on hand for the initial injections.

In rats, iron-dextran complex in very large intramuscular doses was found to induce sarcomas at the injection site.<sup>241</sup> This observation led to the temporary withdrawal of the product between 1960 and 1963. There has

been no evidence of carcinogenicity in man at normal doses.

Iron sorbitex may discolor skin, but less frequently than does iron-dextran complex. A small proportion of the iron in this preparation is ionizable, and if the iron-binding capacity is exceeded, there may be marked systemic reactions, including vasomotor collapse and shock. Many patients complain of a metallic taste which may persist more than a day. At the 100-mg dose, systemic reactions occur in about 5% of patients, and the incidence is higher at greater doses.

### *Response to Therapy*

When specific iron therapy is given, there may be rapid subjective improvement with disappearance or marked diminution of fatigue, lassitude, and other nonspecific symptoms. This response may occur before any evidence of hemoglobin regeneration is observed. One variety of pica, pagophagia or ice-eating, was relieved within one week of therapy.<sup>64</sup>

The earliest hematologic evidence of response to treatment is an increase in the percentage of reticulocytes. The increase generally is slower to appear and not so marked as may be observed following vitamin B<sub>12</sub> therapy of pernicious anemia (page 594). The reticulocytes attain a maximum value on the fifth to the tenth day after institution of therapy, and thereafter gradually return to normal. The maximum value usually ranges from 5 to 10% and is inversely related to the level of hemoglobin.<sup>232</sup> With only slight to moderate degrees of anemia, a pronounced reticulocyte response cannot be expected.

The rate of change in blood hemoglobin levels induced by iron therapy also varies with the degree of anemia (Fig. 17-11). The hemoglobin increases more rapidly at low levels than at high, and it takes about two months to reach normal values, regardless of starting levels. Two simple guidelines that can be used to assess the adequacy of response to therapy are: (1) the hemoglobin should increase to a value half-way between starting level and normal in an average of 18

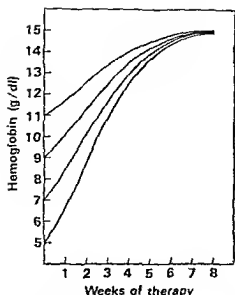


Fig 17-11. The change in blood hemoglobin with time after optimal iron therapy. The more severe the anemia, the more rapid the response. Usually normal values are reached by eight weeks regardless of the starting levels. (From the data of Swan and Jowett<sup>233</sup>)

days; and (2) at three weeks, the hemoglobin should have risen  $59 \pm 17\%$  (mean  $\pm$  S.D.) toward normal.<sup>253</sup> The last observation emphasizes the potential variability of response; to include 95% of normal values (mean  $\pm$  2

S.D.) the increase at three weeks would range from 25 to 93% of the difference between the initial value and normal.

The blood hemoglobin is the most accurate representation of the degree of anemia in iron deficiency. During the response to therapy, the red cell count may temporarily increase to values above normal, but the hemoglobin value lags behind (Fig. 17-12). The red cell indices may remain abnormal for some time after the normal hemoglobin level has been restored (Fig. 17-12).

Of the epithelial lesions in iron deficiency, those affecting the tongue and nails are the most responsive to treatment. After one to two weeks, small regenerating filiform papillae are observed, and this change is also seen on biopsy.<sup>163</sup> By three months the tongue has usually returned to normal, but in patients with severe cases, some atrophy may persist. Koilonychia usually disappears in three to six months, the concavity moving toward the end of the nail as the nail grows. Gastritis and the associated defects in gastric secretion often do not respond to therapy, especially in older adults.<sup>171</sup> In patients less than 30 years of age, gastric acid secretion and normal

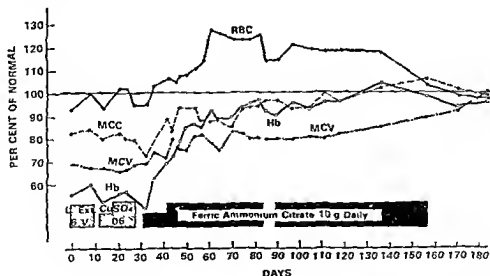


Fig 17-12. Blood changes in a patient with iron deficiency. All values are shown in proportion to the normal for the individual. Note the rapid increase in hemoglobin (Hb) following commencement of iron therapy and then the more gradual rise, the increase in the erythrocyte count (RBC) to values above normal, and the eventual restoration of all values to normal, including those for mean corpuscular hemoglobin concentration (MCC). (From Wintrobe and Beebe,<sup>27</sup> courtesy of the authors and Williams & Wilkins)

epithelial architecture may be restored.<sup>178</sup> Least responsive is sideropenic dysphagia which almost never responds to iron therapy unless dilatation of the upper esophagus also is performed.<sup>141</sup>

In about one third of patients who respond to iron treatment, *relapse* is experienced later, partly because of failure to take medication long enough and partly because of recurrent disease.<sup>60</sup>

### Acute Iron Intoxication

The accidental ingestion of iron compounds by children who have mistaken the tablets for candy is a common pediatric problem. At one time the mortality was as high as 50%. The children who died had swallowed 3 to 10 g or more. Symptoms have been classified in four stages.<sup>246</sup> In stage one, gastrointestinal symptoms predominate (vomiting, diarrhea, melena). Shock may follow, then dyspnea, lethargy, and coma. These events take place in the first six hours following ingestion. In the second stage, lasting from 6 to 24 hours after ingestion, transient improvement occurs and may continue to recovery. In stage three, metabolic acidosis is present. Death may take place 12 to 48 hours after ingestion.

These ill effects are the consequence of the local irritative action of the iron, with mucosal ulceration and bleeding. Capillary dilatation and diapedesis of red cells may occur. Many factors cause the shock, including the absorption of iron in amounts far above the binding capacity of the plasma. Serum iron values as high as 3000 µg/dl have been observed. The cause of the metabolic acidosis is uncertain.<sup>246</sup> Late coma is the result of hypoxia, metabolic disturbances, and hepatic damage.

The introduction of desferrioxamine<sup>231</sup> as a therapeutic agent has greatly modified the outlook.<sup>246,255</sup> This weak base, which has a high specific affinity for iron, can be given orally and intravenously. The molecular complex is small and is excreted quickly by the kidneys. Toxicity is manifested mainly by hypotension, skin rashes, and, when the drug

is ingested in large doses, intestinal irritation.

Aspiration and lavage with 1% sodium bicarbonate should be carried out in any patient who has taken, or even if only suspected of having taken, an overdose of iron salts.<sup>246</sup> Desferrioxamine, 3 g dissolved in 50 ml of distilled water, should be instilled via the nasogastric tube. An intravenous infusion of 1 liter of 5% dextrose in water containing 100 mg desferrioxamine is begun, after drawing blood for measurement of serum iron and total iron-binding capacity. To avoid hypotensive reactions, the rate of infusion should not exceed 15 mg per kg of body weight per hour. The total amount given intravenously will depend on the clinical response. In addition, general supportive measures for shock and acidosis are employed.

If desferrioxamine is not available, calcium disodium EDTA, 80 mg per kg body weight per 24 hours, may be used.<sup>245</sup>

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## *The Anemia of Chronic Disorders and the Sideroblastic Anemias*

Anemia of Chronic Disorders
Symptomatology
Characteristics of the Anemia
Iron Metabolism
Other Biochemical Findings
Kinetic Characteristics
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Diagnosis
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Idiopathic Refractory Sideroblastic Anemia
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Other Drugs

of chronic disorders and increased in sideroblastic anemia.

### Anemia of Chronic Disorders

A mild to moderate anemia frequently accompanies infectious, inflammatory, or neoplastic diseases that persist for more than one or two months (Table 18-1).<sup>8,9,10</sup> Since there are so many such diseases, this type of anemia is very common, perhaps second only to iron-deficiency anemia in overall incidence. The associated biochemical and kinetic alterations form a characteristic pattern, of which the most distinctive feature is the occurrence of hypoferrremia despite abundant quantities of iron in the reticuloendothelial storage depots. The anemia of chronic disorders is defined by the presence of this unique combination of findings.<sup>8,9</sup>

The designation, "anemia of chronic disorders," is far from ideal, but proposed alternatives are even less satisfactory. As defined above, the term is not intended to be used in relation to anemia produced by other mechanisms, such as blood loss, overt hemolysis, or myelophthisis, any one of which may also occur in patients with chronic disorders. Nor is the term meant to refer to anemia in other, equally "chronic" disorders, such as certain renal, hepatic, and endocrine diseases,

**B**OTH the anemia of chronic disorders and the sideroblastic anemias are associated with signs of disturbed iron metabolism, and, in both, hypochromic erythrocytes may be observed. There is rarely any difficulty, however, in distinguishing the two conditions, since, among many other differences, the plasma iron is decreased in the anemia

**Table 18-1. Conditions Associated with the Anemia of Chronic Disorders**

1	Chronic infections <sup>10</sup>
a	Pulmonary infections <sup>36</sup> abscesses amphysema tuberculosis, <sup>12 21</sup> pneumonia
b	Subacute bacterial endocarditis <sup>40</sup>
c	Pelvic inflammatory disease
d	Osteomyelitis
e	Chronic urinary tract infections
f	Chronic fungal disease
g	Meningitis <sup>37</sup>
2	Chronic noninfectious inflammations
a	Rheumatoid arthritis <sup>17 31 49,50 54 43</sup>
b	Rheumatic fever <sup>28</sup>
c	Systemic lupus erythematosus <sup>14</sup>
d	Severe trauma <sup>1 10 20</sup>
e	Thermal injury <sup>15</sup>
f	Myocardial infarction <sup>25 43</sup>
g	Adjuvant disease in rats <sup>38 41</sup>
h	Sterile abscesses <sup>24 25 48</sup>
3	Malignant diseases <sup>3 18 22 27 30 36 42</sup>
a	Carcinoma
b	Hodgkin's disease
c	Lymphosarcoma
d	Leukemia
e	Multiple myeloma
4	Idiopathic <sup>26</sup>

because, in these, the anemia is not necessarily accompanied by the above-described disturbance in iron metabolism. More descriptive, but infrequently used designations include "hypoferremic anemia with reticulo-endothelial siderosis" and "thesauric, hypoferremic anemia."<sup>9</sup>

### Symptomatology

Since this type of anemia occurs in association with so many diseases, the clinical manifestations necessarily vary widely. Usually the signs and symptoms of the underlying disorder overshadow those of the anemia, but, in occasional patients, reduction of the hemoglobin level provides the first evidence of the existence of the primary condition.

### Characteristics of the Anemia

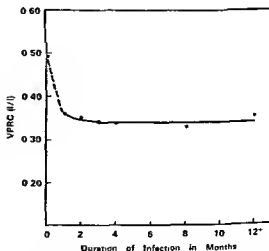
#### Development and Severity

Anemia develops during the first one to two months of illness and thereafter does not

progress (Fig. 18-1).<sup>8</sup> Almost never severely depressed, the VPRC usually is maintained between 0.30 and 0.40 l/l (Fig. 18-2).<sup>8,10</sup> The degree of change may be so modest that the value remains within the low normal range.

Total blood volume was found to be reduced below normal in some patients with anemia due to infection,<sup>10</sup> whereas in others an increase in plasma volume was noted. Such observations imply that the hemoglobin concentration or VPRC may not always reflect the true degree of alteration in the total circulating red cell mass. In most circumstances, however, these measures of concentration are satisfactory indicators of the degree of anemia.

There is a rough correlation between the degree of anemia and the severity of the underlying disease. For example, infections which are accompanied by pronounced fever, chills, and suppuration are associated with more severe anemia than are those with fewer systemic manifestations.<sup>8</sup> There is some evidence that the greater the number of organisms in a wound, the more severe the anemia. Correlation between the severity of the anemia and the activity of rheumatoid arthritis, as judged by fever, severity of joint swelling and inflammation, and the sedimentation



**Fig 18-1** The development of anemia, as measured by the volume of packed red cells (VPRC), in 50 patients with chronic infections (From Cartwright,<sup>8</sup> courtesy of the author and Henry M. Stratton, Inc.)

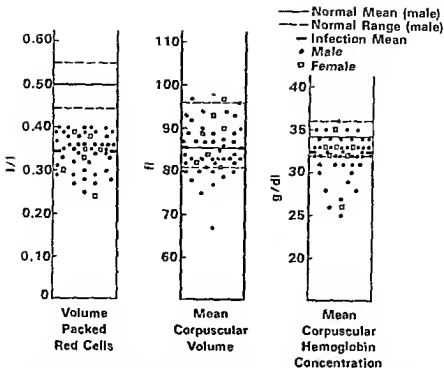


Fig 18-2. Degree and type of anemia as indicated by the volume of packed red cells, the mean corpuscular volume and the mean corpuscular hemoglobin concentration in 50 patients with anemia due to infection (From Cartwright and Wintrobe<sup>10</sup> courtesy of the authors and Year Book Medical Publishers, Inc.)

rate, also has been observed.<sup>31</sup> In patients with malignant disease, anemia is more severe when there are widespread metastases than when the disease is localized; however, the development of anemia does not require invasion of the bone marrow.<sup>30,42</sup> The anemia of malignant disease may also take other forms (see sideroblastic anemia, page 685, and myelophthisic anemia, Chapter 57).

### Morphologic Features

The anemia usually is normocytic and normochromic (Fig. 18-2). However, hypochromia (MCHC 26 to 32 g/dl) has been observed in 23 to 50% of patients with chronic infection, 50 to 100% of patients with rheumatoid arthritis, and 44 to 64% of patients with cancer.<sup>8</sup> Hypochromia may be observed even though the VPRC remains within normal limits.<sup>36</sup> Microcytosis is encountered less often than hypochromia and, when present, is usually not as great as is commonly found in iron-deficiency anemia. Values for MCV below 72 fl are rare.<sup>8</sup>

Anisocytosis of moderate degree may be detected, but poikilocytosis is slight. As a rule, few other changes in the red cells are observed. Evidence of attempted regeneration, such as polychromatophilia or the presence of nucleated red cells in the circulation, is conspicuous by its absence. Generally, the reticulocytes are normal or reduced in number,<sup>11</sup> but sometimes they may be slightly increased.<sup>51</sup>

### Iron Metabolism

Characteristically, the serum iron is decreased, total iron-binding capacity is reduced, and transferrin saturation is subnormal (Fig. 18-3; also see Figs. 16-2 and 16-3, page 628).<sup>2,8</sup> In patients with infection, hypoferrinemia develops early in the course of the illness, frequently within 24 hours, and is observed even in acute, self-limited febrile diseases or following a single injection of typhoid vaccine.<sup>8,10,11</sup> Similarly, a rapid decrease in plasma iron follows the injection of either bacterial endotoxin,<sup>33</sup> or

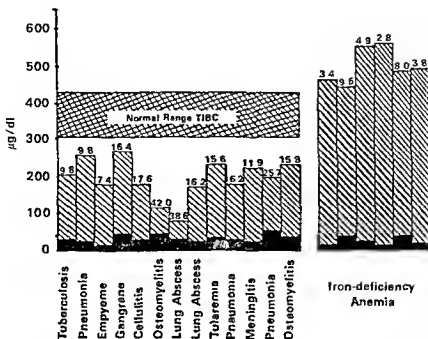


Fig. 18-3 The serum iron and the total iron binding capacity of the serum in 13 patients with anemia associated with infection as compared with the normal and in six patients with iron-deficiency anemia.

Solid areas represent serum iron. Hatched areas represent the unsaturated iron-binding capacity. The total height of each column represents the total iron binding capacity of the serum. The figures above the columns represent the percent saturation (SI/TIBC). (From Cartwright et al.<sup>11</sup> courtesy of authors and *Journal of Clinical Investigation*.)

a substance similar to the "endogenous pyrogen" formed by neutrophilic leukocytes.<sup>46</sup> When the infection is of short duration, the serum iron returns to normal and anemia does not develop; in prolonged illnesses, the serum iron level remains low as long as the disease is active. When the disorder subsides, anemia often is relieved before the serum iron returns to normal. The degree of hypoferrinemia is related to the severity of the underlying illness.<sup>2,6,17,31</sup>

In bone marrow aspirates stained for iron, sideroblasts are found to be reduced to 5 to 20% of the total normoblasts (normal, 30 to 50%). In contrast, hemosiderin within macrophages usually is increased (Fig. 18-4); exceptions probably represent cases complicated by iron deficiency.

Absorption of iron from the gastrointestinal tract is reduced.<sup>8,11,13,27</sup> This abnormality appears to result from failure of the iron to

pass from the intestinal mucosal cell into the body.<sup>52</sup>

### Other Biochemical Findings

The concentration of free protoporphyrin in the erythrocytes (FEP) tends to increase in patients with chronic disorders<sup>10,32</sup> (Fig. 18-5). Levels of 36 to 634 µg per dl packed red cells (mean 180 µg), compared with normal values of 14 to 79 µg (mean 36 µg), were found in patients with infections.<sup>8</sup> The concentration of free erythrocyte coproporphyrin (FEC) was also increased, but only approximately twofold, rather than fivefold, and urinary excretion of coproporphyrin was increased. Unlike the change in serum iron, the FEP increases slowly and does not become clearly abnormal until significant anemia has developed.

In most patients with the anemia of

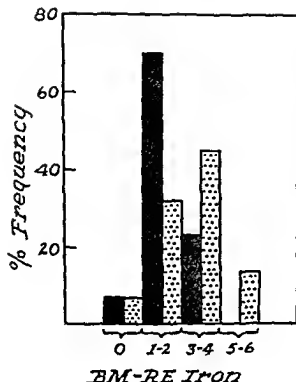


Fig 18-4. RE cell iron in bone marrow in 95 patients with chronic infection (dotted bars) compared with that found in 82 normal subjects (solid bars). The quantity present ranged from none (0) to heavy deposits (5 to 6+) (From Cartwright,<sup>8</sup> courtesy of the author and Henry M. Stratton Inc.)

chronic disorders, hypercupremia occurs. The levels associated with infection ranged from 118 to 267  $\mu\text{g/dl}$  (mean 191  $\mu\text{g/dl}$ ) compared with the normal range of 81 to 147  $\mu\text{g/dl}$  (mean 114  $\mu\text{g/dl}$ ).<sup>8,20</sup> This increase is due mainly to an increase in the plasma copper protein, ceruloplasmin (page 150). The hypercupremia precedes the development of anemia and, as the infection subsides, the copper content of the blood returns to normal more rapidly than does iron content of the serum.

#### Kinetic Characteristics

Ferrokinetic studies in patients with chronic infections,<sup>7,11,27,34</sup> rheumatoid arthritis,<sup>17,52,61</sup> and various malignant diseases<sup>30,42</sup> have shown that (1) the rate of disappearance of iron from the plasma is rapid, (2) the plasma iron transport rate is normal or slightly increased, (3) the uptake

of iron into erythrocytes and the amount of iron turning over through red cells daily are normal or increased, and (4) the fraction of red cells renewed daily is increased.<sup>8</sup> These findings indicate that erythropoiesis is normal or increased to only a moderate degree.

The rate of erythrocyte destruction is accelerated.<sup>7</sup> Erythrocyte survival, measured by the Ashby and <sup>51</sup>Cr-tagging procedures, is shortened modestly.<sup>8</sup> However, the usual manifestations of increased blood destruction, such as increased serum bilirubin and increased urobilinogen excretion, generally are absent.<sup>11,51</sup> The survival of cells from patients with arthritis, when transfused into normal subjects, has been found to be normal, and the survival of normal red cells in the circulation of patients with arthritis is less than normal.<sup>50</sup> These observations indicate that the accelerated breakdown of the red cells can be attributed to an extracorporeal factor.<sup>17</sup>

In summary, the kinetic data suggest that anemia develops because the bone marrow fails to increase red cell production sufficiently to compensate for a mild decrease in the life span of the red cells.

#### Pathogenesis

Three factors have been implicated in the pathogenesis of the anemia: (1) decreased erythrocyte life span, (2) impaired marrow response, and (3) unpaired flow of iron from reticuloendothelial stores to plasma.<sup>8</sup>

#### Erythrocyte Life Span

The kinetic studies cited in the preceding paragraphs indicate the presence of an extracorporeal factor acting to shorten erythrocyte survival. The nature of this factor remains unknown; however, a number of mechanisms have been suggested.<sup>8</sup> In animal studies, sterile, cell-free extracts of the viable portions of malignant tumors were found capable of hemolyzing red cells.<sup>55</sup> It also was shown that in vivo irradiation of normal or carcinomatous tissue can initiate a hemolytic mechanism.<sup>54</sup> Such observations led to the

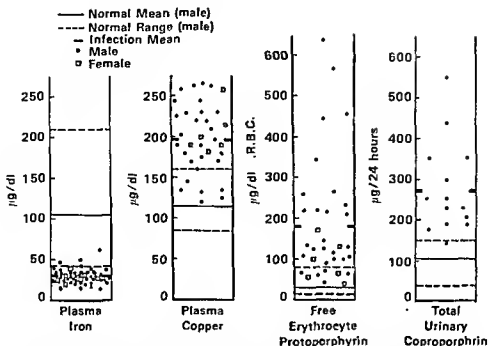


Fig 18-5 The severe hypoferrremia increased plasma copper and increased erythrocyte protoporphyrin and urinary coproporphyrin in anemia associated with infection as indicated by a study of 50 cases (From Cartwright and Winrobe<sup>10</sup> courtesy of the authors and Year Book Medical Publishers, Inc.)

suggestion that hemolysins elaborated by the tumor may contribute to the anemia in cancer. Other studies suggested that vascular injury may be a factor in the pathogenesis of the erythrocyte destruction.<sup>47</sup> According to the latter hypothesis, red cells are destroyed as the result of leakage into the tumor. Still other studies have sought to discover the possible role of immune mechanisms, such as elaboration of autoantibodies by the malignant cells.<sup>22</sup> Bacterial toxins have been implicated as hemolytic factors in infections.<sup>34</sup> None of these explanations is entirely satisfactory. Furthermore, the similarity of findings in animals with sterile and bacterial abscesses and those in patients with the many and various diseases associated with the anemia of chronic disorders suggests that the mechanism may be the same in all these situations. It is possible that these diseases result in nonspecific stimulation of the reticulo-endothelial system, one consequence of which is an increase in this system's activity in destroying red cells.<sup>8,9</sup> Such a nonspecific

activation of macrophages (page 322) can be induced by a variety of stimuli, a phenomenon related to "cellular immunity."<sup>35</sup>

### Impaired Marrow Response

Normal bone marrow, being capable of a six- to eightfold increase in the red cell production rate, could easily compensate for the modest reduction in erythrocyte survival. Its failure to do so in the anemia of chronic disorders suggests that impaired production capacity is of fundamental importance in pathogenesis. The marrow response to anemia is under the control of erythropoietin (Chapter 4). Low serum erythropoietin levels in relation to the degree of anemia have been found in both man and experimental animals with this type of anemia.<sup>55,59</sup> Furthermore, such animals produced less than normal amounts of erythropoietin in response to hypoxia.<sup>37a</sup> However, if erythropoietin is administered, or if its endogenous elaboration is stimulated with cobalt or hypoxia, the



marrow is capable of responding to it.<sup>24,37a</sup> Thus, the defect in production appears to result from failure to secrete erythropoietin rather than from an inability of the marrow to respond to the hormone. It has been suggested that these observations reflect subtle alterations in control mechanisms, ie, "turning the hematologic thermostat down a bit," for the purpose of diverting substrates normally utilized in hemoglobin production to other, more critical, channels.<sup>9</sup>

### *Defective Iron Metabolism*

The alterations in iron metabolism appear to result from a block in the flow of iron from RE cells to plasma.<sup>8,16</sup> This block has been identified in several ways. With an <sup>59</sup>Fe label, reutilization of iron from effete red cells or hemoglobin solutions was found to be impaired.<sup>8,16,27,35,48,61</sup> A similar defect was observed when iron dextran was the iron source.<sup>4</sup> Such observations may be partly explained by dilution of the isotope by the increased stores of the reticuloendothelial cells,<sup>37</sup> but this explanation is not entirely satisfactory because it cannot account for the observation that the total amount of iron released following administration of damaged erythrocytes is subnormal.<sup>44</sup> A relatively simple way of demonstrating the reticuloendothelial iron block *in vivo* is to administer nicotinic acid intravenously, which induces a minor episode of hemolysis. In normal subjects the injection was followed by a prompt increase in serum iron; in patients with rheumatoid arthritis the increase was subnormal.<sup>45</sup>

As one consequence of RE iron blockade, erythropoiesis becomes limited by the insufficient iron supply,<sup>2</sup> accounting for the decreased marrow sideroblasts, the increased FEP, and the occasional occurrence of hypochromia. The nature of the relationship between the iron block and the defect in erythropoietin secretion requires further study. However, the observation that the anemia can be relieved by erythropoietin administration suggests that the hormone directly or indirectly relieves the block.<sup>9</sup>

One possible explanation for the abnormalities in iron metabolism is that they represent a mechanism which has evolved to defend the host against bacterial invasion.<sup>6a,57,60</sup> The low plasma iron value serves to inhibit bacterial growth. Furthermore, the increased RE iron may be useful in detoxifying virulent bacterial products.

### *Diagnosis*

In most instances, patients with anemia due to chronic disorders do not appear with anemia as the sole manifestation of illness. They are likely to complain of fever, chills, joint pains, weight loss, or other manifestations of the underlying illnesses. Only in the rare instances in which no specific signs or symptoms are present will difficulties in establishing the diagnosis arise. Under such circumstances, careful study of iron metabolism, as outlined in Chapter 16, should suggest the anemia of chronic disorders and distinguish it from that due to iron deficiency.

The next and most challenging step is the definition of the underlying disease. Little help in this direction is obtained from further study of the blood, for the anemia of chronic disorders is a nonspecific sign of disease, comparable to neutrophilic leukocytosis or accelerated erythrocyte sedimentation.

### *Treatment*

Correction of the anemia depends on relief of the primary disorder. Blood transfusion may be useful as a temporary measure, but the anemia is rarely so severe as to justify the expense, risks, and discomforts of transfusion. The anemia usually is not progressive and there is no evidence that it is harmful to the patient. General measures, as outlined in Chapter 13, should be employed. Vitamin B<sub>12</sub> and folic acid are of no value but, if there is an associated iron deficiency, as sometimes occurs, particularly in rheumatoid arthritis,<sup>59</sup> iron therapy may relieve the anemia to some degree.<sup>50</sup> Parenteral iron therapy should be avoided since, in the absence of associated iron deficiency, the tissues already are amply

supplied with iron. Iron given intravenously is rapidly shunted to the tissues without influencing the plasma iron level more than temporarily and without relieving the anemia<sup>11,62</sup>

Although cobalt (page 153), in the form of cobaltous chloride, 100 to 300 mg per day taken by mouth, will partially relieve the anemia associated with infection,<sup>5,19,62</sup> loss of appetite and other gastrointestinal symptoms are likely to occur, the effect of the treatment

disappears when use of the drug is discontinued, and no real benefit is achieved.

## Sideroblastic Anemias

The sideroblastic anemias are a heterogeneous group of disorders<sup>139,158</sup> that are characterized by the presence of excessive iron deposition within the mitochondria of normoblasts<sup>105,129,139,172,183</sup> (Fig. 18-6). Because of their characteristic perinuclear dis-



Fig 18-6 Excessive iron deposits in the mitochondria within normoblasts of a patient with sideroblastic anemia (Courtesy of WN Jensen)

tribution, these iron-laden mitochondria account for the so-called "ringed" sideroblast, a nucleated red cell in which Prussian blue-positive granules form a full or partial ring around the nucleus<sup>109</sup> (Plate X).

Mitochondrial iron excess appears to be a consequence of defective heme synthesis. Additional evidence of defective heme synthesis in sideroblastic anemia is found in the circulating red cells, which usually are hypochromic and microcytic. The degree of hypochromia and microcytosis varies considerably from one form of sideroblastic anemia to another. Often there is pronounced *dimorphism*, a hypochromic and/or microcytic population of cells existing side by side with a normal or even macrocytic one.

In addition to these morphologic findings, the sideroblastic anemias are characterized by a great increase in total body iron. Serum iron is increased, often to the point of complete saturation of transferrin. Excessive amounts of iron are deposited in cells of the reticulo-endothelial system and in the parenchymal cells of various organs. In some instances the excess iron appears to interfere with organ function, especially that of the liver, pancreas, and heart, resulting in the clinical picture of hemochromatosis.

These anemias are characterized kinetically by ineffective erythropoiesis.<sup>134,171,182</sup> Erythroid hyperplasia of the bone marrow is accompanied by a normal or only slightly increased reticulocyte count. The plasma iron transport rate is markedly increased, but red cell iron utilization is impaired. Red cell survival, as measured with the usual random labels (page 197), tends to be normal or only slightly reduced. There may be slight hyperbilirubinemia and an increase in urobilinogen excretion as the result of an increase in the erythropoietic component of the "early-labeled" bilirubin peak.<sup>102</sup> These findings suggest that many of the red cells formed from the maturation of ringed sideroblasts are essentially nonviable. Their destruction within the marrow or very shortly after their release accounts for the kinetic abnormalities.

Other terms used more or less synonymously with sideroblastic anemia include

*sideroachrestic anemia*,<sup>138</sup> and *iron-loading anemia*.<sup>113,130</sup> In addition, many of the cases formerly referred to as "*refractory anemia with hyperplastic bone marrow*"<sup>175</sup> probably belong in this category.

### Classification

There are many sideroblastic anemias and several classifications have been proposed.<sup>138,139,158</sup> For certain forms of sideroblastic anemia, the cause and pathogenesis seem reasonably well delineated. In others, the diseases must be defined on the basis of a combination of descriptive clinical, morphologic, and biochemical features. Consequently, continued refinement of any proposed classification scheme is to be expected.

In Table 18-2, the sideroblastic anemias have been divided into two major groups, depending on whether the illness appears to be inherited or acquired. Within the inherited group, an X-linked, often pyridoxine-responsive form has been delineated, which probably represents a congenital defect in the enzyme ALA synthetase.<sup>176</sup> In one family, a similar illness appeared to be inherited as an autosomal recessive trait.<sup>117</sup> In some patients, the finding of markedly increased erythrocyte coproporphyrin combined with reduced values for free erythrocyte protoporphyrin suggested that the enzyme coproporphyrinogen oxidase was defective.<sup>125,138</sup>

The acquired sideroblastic anemias are considerably more common than the inherited varieties. These may be subdivided on the basis of whether the anemia is of un-

**Table 18-2. Classification of the Sideroblastic Anemias**

A Hereditary sideroblastic anemia	
1	X linked
a	ALA synthetase deficiency <sup>176</sup>
b	(P) coproporphyrinogen oxidase deficiency <sup>125</sup>
2	Autosomal recessive <sup>117</sup>
B Acquired sideroblastic anemia	
1	Idiopathic refractory sideroblastic anemia <sup>145</sup>
2	Complicating other diseases <sup>139, 156</sup> (Table 18-3)
3	Associated with drugs or toxins (Table 18-4)

known cause (idiopathic) or associated with toxins or underlying diseases (Table 18-2).

### Hereditary Sideroblastic Anemia

This disorder was probably first described in 1946, under the name "hereditary (sex-linked) anemia," by Rundles and Falls.<sup>165</sup> Ten years later, Harris and coworkers reported the first case of so-called *pyridoxine-responsive anemia* in man.<sup>135</sup> Shortly thereafter, a member of one of the families studied by Rundles and Falls was found to be responsive to pyridoxine.<sup>106</sup>

Many of the patients with hereditary sideroblastic anemia have been reported as patients with pyridoxine-responsive anemia.<sup>134</sup> However, the degree to which hereditary sideroblastic anemia responds to pyridoxine varies considerably. In some patients, anemia has been corrected completely, others responded partially, and still others did not respond at all.

It is possible that these variations indicate fundamental differences in the nature of the disease; on the other hand, varying degrees of pyridoxine responsiveness have been observed within members of the same kindred.<sup>108,151</sup> Furthermore, responsiveness may decrease with subsequent courses of pyridoxine in the same patient.<sup>134</sup> Except for the response to therapy, there are no distinct clinical, hematologic, or biochemical differences between pyridoxine-responsive and refractory forms.

### Hereditary Pattern

Most patients with hereditary sideroblastic anemia have been males. Of 72 patients with "pyridoxine-responsive anemia," 22 (15 families) related a positive family history.<sup>134</sup> Only one of the 22 was female. Male subjects also predominated among the remaining patients. This male preponderance suggests that the disorder is inherited most commonly as an X-linked recessive trait. Several kindreds supporting such a mode of inheritance have been reported (Fig. 18-7).<sup>108,151,152,160,165,180</sup>

In such families, usually only male hemi-

zygotes have been anemic; however, morphologically abnormal erythrocytes have been found in female carriers. In exceptional families, the female carriers were moderately anemic,<sup>148,169,170</sup> and, in at least one such family, the abnormal red cell population appeared to be so severely lacking in hemoglobin that the trait would probably have been lethal in males.<sup>145</sup> In this family, the X-linked red cell antigen, Xg<sup>a</sup>, was found on the abnormal cells only; normal cells were Xg<sup>a</sup>-negative. These observations were interpreted as supporting an X-linked mode of inheritance, with mosaicism in the female patient on the basis of X-inactivation. A similar distribution of the Xg<sup>a</sup> antigen could not be demonstrated in two other families<sup>160,170</sup>; however, the abnormal cell populations were not as severely affected, and appeared not to have been completely separated from the normal one for study.

In one reported family, a brother and sister were affected equally, suggesting autosomal inheritance.<sup>117</sup>

### Clinical Picture

The anemia may be apparent at birth or in infancy, but the onset most often occurs in young adulthood.<sup>134</sup> Exceptionally, the anemia is not discovered until after the subject has reached the age of 60 years. Such variations may occur even within a single kindred.<sup>151</sup> The factors that delay the onset of this inherited condition have not been completely defined. Unusually high dietary or medicinal pyridoxine intake may be one such factor.<sup>163</sup> The degree of iron overload may be another.<sup>176,180</sup>

In addition to the signs and symptoms of anemia, most patients exhibit manifestations of iron overload. Mild to moderate enlargement of the liver and spleen is common; however, liver function usually is normal or only mildly disturbed. Liver biopsy reveals excessive iron in both parenchymal and RE cells with a variable amount of fibrosis.<sup>134</sup> Frank clinical diabetes or abnormal glucose tolerance may be detected in as many as one third of the patients. Occasionally, abnormal

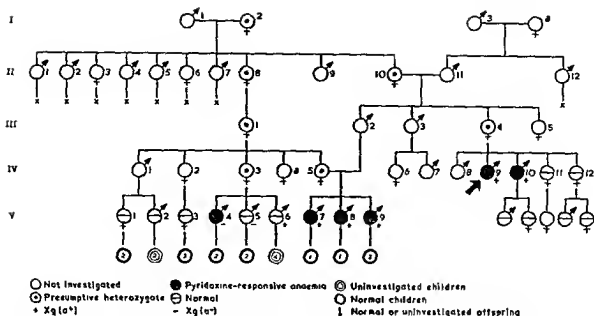


Fig. 18-7 Pedigree of a family with hereditary sideroblastic anemia (From Elves et al.<sup>123a</sup> courtesy of the authors and Journal of Medical Genetics)

skin pigmentation is observed. The most dangerous manifestations of iron overload are disturbances of cardiac rhythm; fortunately, these tend to be relatively uncommon and to occur late in the course of the disease.

Signs and symptoms of nutritional deficiency are absent. In particular, neurologic and cutaneous manifestations of vitamin B<sub>6</sub> deficiency (page 144) are not observed.

### Laboratory Findings

The anemia usually is severe (average blood hemoglobin 6.5 g/dl), and hypochromia and microcytosis are pronounced (Table 16-3, page 626).<sup>134</sup> Anisocytosis, poikilocytosis, target cells, and basophilic stippling are prominent findings on blood smear (Fig. 18-8), and erythrocyte dimorphism is common. Leukocytes and platelets usually are normal. Erythroid hyperplasia is found on marrow examination, and maturation usually is normoblastic; occasionally, megaloblastic changes are observed,<sup>134</sup> probably because of complicating folate deficiency.

Transferrin saturation almost invariably is increased. Excessive hemosiderin stores are found in marrow. Ringed sideroblasts consti-

tute about 10 to 40% of the normoblasts. The ringed forms usually are found in the polychromatophilic or orthochromic stages of normoblast maturation.<sup>133</sup>

Kinetic evidence of ineffective erythropoiesis usually can be demonstrated.

In about one third of the patients, abnormal tryptophan metabolism, as indicated by the excessive urinary excretion of xanthurenic and/or kynurenic acid following a tryptophan load, has been observed. These abnormalities have been interpreted as evidence of deficiency or abnormal metabolism of vitamin B<sub>6</sub>. However, they may also occur nonspecifically as the result of induction of hepatic tryptophan pyrrolase.<sup>101</sup>

The free erythrocyte protoporphyrin (FEP) level tends to be reduced or low normal.<sup>134,161,175</sup> In isolated red cell populations from a female patient, the FEP was very low in the microcytic cells and normal in the normocytic ones.<sup>145</sup> Erythrocyte coproporphyrin (FEC) usually is normal; however, in an unusual patient reported by Garby and coworkers, the low FEP was accompanied by very high FEC values.<sup>125</sup> Heilmeyer, drawing on clinical material from all over Europe, found porphyrin alterations similar to those

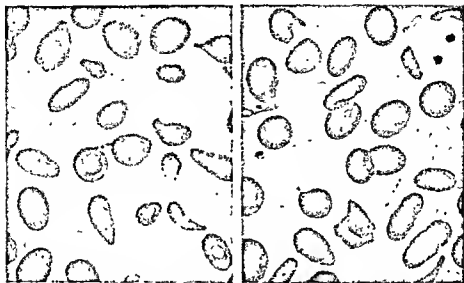


Fig. 18-8 Blood smears of a patient (E O R) with pyridoxine-responsive anemia. The smear on the right was made after pyridoxine 100 mg daily, had been given intravenously for 30 days and the hemoglobin had risen from 7.7 to 9.8 g/dl (From Raab et al,<sup>141</sup> courtesy of the authors and Henry M. Stratton, Inc.)

reported by Garby and associates in six additional patients, all males.<sup>138</sup>

### Treatment

Pyridoxine therapy should be tried in all patients. Doses of 50 to 200 mg/day usually are administered. There is no convincing evidence that the parenteral route is more effective than the oral one. These doses are very large as compared with the estimated adult daily requirement for vitamin B<sub>6</sub> of 1.5 to 2 mg. As previously noted, the response to pyridoxine is variable. In somewhat less than half of the patients an "optimal response" is observed: reticulocytosis occurs, the blood hemoglobin returns to normal or near normal levels, the serum iron decreases, the FEP increases,<sup>148,161</sup> and abnormalities in tryptophan metabolism are corrected. However, morphologic abnormalities of the red cells never completely disappear (Fig. 18-8).<sup>134</sup> Of the patients in whom optimal response is not obtained, some experience distinct, but suboptimal improvement when pyridoxine is given, and the blood hemoglobin stabilizes at less than normal levels.

Still others have not responded at all, including all of those with high FEC levels.<sup>133</sup>

Once the maximal effect of pyridoxine has been achieved, continued maintenance doses are required. Relapse usually will follow within several months after discontinuing treatment.<sup>134,161</sup> After relapse, it may be possible to induce additional remissions with pyridoxine, but in some instances the response was less satisfactory than with the first course of therapy.<sup>134</sup> In one patient, responsiveness to pyridoxine was restored by administration of the amino acid, L-tryptophan.<sup>142</sup>

If megaloblastic changes are present, folic acid should be added to the regimen. Usually such therapy leads to normoblastic maturation, suboptimal reticulocytosis, and an increase in hemoglobin levels. Of special interest is an occasionally observed response to Valentine's crude liver extract, possibly to an indole contained therein.<sup>134,142</sup> In one report, a patient who responded to an unidentified material in human plasma was described.<sup>124</sup> Androgens also may be effective.<sup>126</sup>

In several patients, the effect of removal of excess body iron was assessed.<sup>134,176,180</sup>

The results were variable. In general, after excess iron was removed, pyridoxine was still required, but at times somewhat greater hemoglobin levels could be maintained. Serum iron values were clearly lower,<sup>134</sup> and *in vitro* heme synthesis was improved.<sup>176</sup> Even if there is no hematologic improvement, the risk of hemochromatosis is eliminated. Thus, in patients who can tolerate it, removal of the excess iron should be seriously considered.

The course is variable and the prognosis must be guarded. Those patients who respond well to pyridoxine may live comfortably for many years.<sup>134</sup> Others have died after having become refractory to therapy, usually because of cardiac arrhythmias, liver failure, bone marrow failure, or infections.

### Pathogenesis

Patients with hereditary sideroblastic anemia exhibit many of the features of experimentally induced vitamin B<sub>6</sub> deficiency in swine (Chapter 4). They differ in that (1) evidence of nutritional deprivation is lacking, (2) unusually large amounts of vitamin B<sub>6</sub> (pyridoxine) are required for correction of the syndrome, and (3) response is incomplete.

Pyridoxal-5-phosphate, the coenzyme form of vitamin B<sub>6</sub>, is required for the synthesis of delta-aminolevulinic acid.<sup>167</sup> This reaction occurs within mitochondria and is the first step in porphyrin biosynthesis (Chapter 4). Indirect evidence suggests that hereditary sideroblastic anemia arises from a genetic defect in this step.<sup>146</sup> Such a defect could account for the anemia, the hypochromia, the reduced FEP, the mitochondrial iron accumulation, and the response to pyridoxine. Presumably the genetic lesion affects the enzyme, ALA synthetase.<sup>176</sup> Although it may seem peculiar that large doses of a cofactor could compensate for an enzymatic deficiency, there are other inherited disorders in which pyridoxine seems to exert a similar effect, including "pyridoxine-dependent" seizures in children, and cystathioninuria.<sup>146,168</sup> It is to be expected that, as in other inherited enzymatic deficiencies, several

allelic variants may exist, accounting for the variability in clinical severity and pyridoxine responsiveness.

It seems likely that the children, described above, with high FEC values have a defect of a different sort, namely, in the conversion of coproporphyrinogen to protoporphyrin (the coproporphyrinogen oxidase reaction).<sup>125,138</sup> This step does not require pyridoxal phosphate as a cofactor, and such children are refractory to pyridoxine therapy.

### Idiopathic Refractory Sideroblastic Anemia (IRSA)<sup>145</sup>

First described by Bjorkman<sup>107</sup> and by Dacie and associates,<sup>119</sup> this form of sideroblastic anemia has also been called "refractory normoblastic anemia,"<sup>119</sup> "anemia refractoria sideroblastica,"<sup>136,138</sup> "idiopathic ineffective erythropoiesis,"<sup>117</sup> and "primary acquired refractory sideroblastic anemia."<sup>139</sup> Although accurate incidence figures are not available, the illness appears to be uncommon but not rare.<sup>145</sup> The cause and pathogenesis are unknown, but the descriptive features appear to be sufficiently characteristic to define the illness as a distinct entity.

### Clinical Features

IRSA is a disease of older adults. In a group of 61 patients, the average age at the time of presentation was 66 years,<sup>145</sup> and very few patients less than 50 years of age have been reported. The anemia develops insidiously and often is discovered at the time of a routine examination or in association with an unrelated complaint. Some patients complain of mild symptoms of anemia. The physical examination often reveals no abnormality except for slight pallor, but hepatosplenomegaly of mild degree is found in about 40% of the patients.

### Laboratory Findings

The anemia usually is moderate in degree (mean VPRC 0.27 l/l) and normocytic or macrocytic (mean MCV 100 fl) in type (Fig.

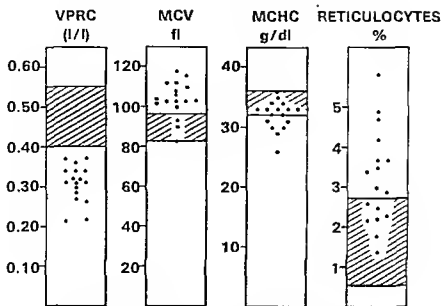


Fig 18-9. Characteristics of the anemia in 17 patients with IRSA.<sup>145</sup> The anemia was usually macrocytic and either normochromic or slightly hypochromic. The reticulocyte count was within the normal range or slightly above it. The normal range (mean  $\pm$  2 SD) is indicated by the shaded areas. VPRC: Volume of packed red cells; MCV: mean corpuscular volume; MCHC: mean corpuscular hemoglobin concentration. (From Kushner et al,<sup>145</sup> courtesy of the authors and William & Wilkins Co.)

18-9) The MCHC is normal or slightly reduced, but a small population of hypochromic cells is found on blood smear in all the subjects. A particularly characteristic finding is the presence of occasional, heavily stippled, hypochromic cells. Leukocyte and platelet counts usually give values within the normal range, but modest leukopenia and/or thrombocytopenia may be found. Leukocyte alkaline phosphatase is reduced in about half of the patients.

Erythroid hyperplasia (average M:E ratio, 1.1:1) is found on bone marrow examination. A feature which may be helpful in distinguishing IRSA from erythroleukemia is the lack of PAS-positive material in the normoblasts. Megaloblastic changes, correctable with folate administration, are found in 20% of patients with IRSA. Marrow hemosiderin is almost always increased and ringed sideroblasts comprise 43 to 95% of the normoblasts. In contrast to hereditary or secondary sideroblastic anemias, the early (basophilic) normoblasts may be ringed.<sup>133</sup>

Transferrin saturation is increased in nearly all patients and exceeds 90% in about one third of the patients.<sup>145</sup> Marked deposition of iron is found in the liver, but hepatic dysfunction is rare.

Free erythrocyte protoporphyrin (FEP) characteristically is increased to 46 to 300  $\mu\text{g/dl}$  (normal 16 to 36  $\mu\text{g/dl}$ ). In one patient very high values were found (1700  $\mu\text{g/dl}$ ), and this abnormality was associated with dermal photosensitivity.<sup>164</sup> If pyridoxine is administered, a further increase in FEP occurs.

There may be modest increases in serum bilirubin (no greater than 2.0 mg/dl) and in urobilinogen excretion. The kinetic pattern is that found with ineffective erythropoiesis.

#### Treatment and Prognosis

A therapeutic trial of pyridoxine, 50 to 200 mg per day, is indicated, but most patients do not respond,<sup>145</sup> and in those who do the response is minimal.<sup>139</sup> However, about half



of the patients with IRSA are not severely incapacitated by their anemia. Furthermore, there may be no progression for a number of years. In such patients, no therapy is required. On the other hand, if the VPRC tends to fall to levels at which significant cardiovascular symptoms occur, treatment may be necessary. Sometimes, transfusion of packed red cells is the only means of relieving the anemia, but transfusion therapy should be kept to a minimum since it will add to the iron overload. A proportion of patients respond to large doses of androgens (eg, 50 to 300 mg of oxymetholone per day),<sup>145</sup> and a trial of such therapy is warranted in patients who require transfusions.

The median survival of patients with IRSA was 10 years<sup>145</sup>; it was longer in patients who did not require transfusion (15 years) than in those who did (eight years). In some cases (about 7%), the terminal events were marked by the onset of leukemia, usually of the acute myelomonoblastic type.

### **Etiology and Pathogenesis**

The cause of IRSA remains unknown. Because of the age distribution and lack of affected relatives, it is assumed to be an acquired disorder. Suggested etiologic factors include neoplasia<sup>121</sup>; hypersensitivity reactions to drugs, toxins, or environmental pollutants; deficiency of an unknown nutrient; and somatic mutation.<sup>145</sup> The available data do not affirm the etiologic role of, or exclude the possibility of, any of these. Because of the observed increase in FEP, it has been proposed that the *heme synthetase reaction*, in which protoporphyrin is converted to heme, is impaired.<sup>138</sup> In one unique patient, the value for FEP was unusually high and heme synthetase activity was reduced in reticulocytes, possibly because of deficiency of a hypothetical cofactor.<sup>164</sup> However, in other patients with IRSA, no defect in reticulocyte heme synthetase could be found.<sup>145,173</sup> Indeed, assays of the entire heme biosynthetic pathway in circulating erythrocytes gave normal values.<sup>145,173</sup> On the other hand, in vivo studies of incorporation of glycine and

valine into hemoglobin in patients with IRSA suggested that globin synthesis was normal and that heme synthesis was defective.<sup>177</sup> Additional indirect evidence supporting such a defect is the observation that heme-free  $\alpha\beta$ -globin dimers could be demonstrated in reticulocytes from patients with IRSA and that the addition of heme in vitro removed the free dimer and greatly stimulated globin synthesis.<sup>181</sup>

### **Sideroblastic Anemia as a Complication of Other Diseases**

So-called "secondary" or "symptomatic" sideroblastic anemia has been observed in association with a wide variety of illnesses (Table 18-3). This type of anemia is common, but poorly characterized and incompletely understood. In most instances the clinical picture is dominated by signs and symptoms of the underlying disease. The presence of anemia, occasional hypochromic cells on blood smear, and ringed sideroblasts in the marrow establishes the diagnosis. In some patients with leukemia,<sup>114</sup> myeloma,<sup>102,114</sup> or

**Table 18-3. Diseases Which May Be Complicated by Sideroblastic Anemia**

<b>A Diseases of the blood and blood forming organs</b>	
1	Leukemia <sup>114 170</sup>
2	Polycythemia vera <sup>139 156</sup>
3	Myelofibrosis <sup>139 156</sup>
4	Hemolytic anemia <sup>156 159</sup>
5	Pernicious anemia and other megaloblastic anemias <sup>154</sup>
<b>B Neoplastic diseases</b>	
1	Hodgkin's disease <sup>119</sup>
2	Other lymphomas <sup>109</sup>
3	Carcinoma <sup>139 147 154</sup>
4	Multiple myeloma <sup>102 114 144</sup>
<b>C Inflammatory diseases</b>	
1	Rheumatoid arthritis <sup>139 156</sup>
2	Polyarteritis nodosa <sup>154</sup>
3	Infection <sup>109 110 146</sup>
<b>D Miscellaneous disorders</b>	
1	Myxedema <sup>139 154</sup>
2	Thyrotoxicosis <sup>139</sup>
3	Uremia <sup>122 139</sup>
4	Erythropoietic porphyria <sup>131 137</sup>
5	Porphyria cutanea tarda <sup>129</sup>

metastatic carcinoma,<sup>147</sup> sideroblastic anemia was present for several months before the underlying disease became apparent.

In general the number of ringed sideroblasts and the degree of hypochromia, hyperferremia, iron overload, and ineffective erythropoiesis are less than in other sideroblastic anemias. Complicating megaloblastosis with decreased serum folate levels is common.<sup>150</sup>

The treatment, course, and prognosis are related to the nature of the associated disease. When the disease can be cured or relieved, the sideroblasts may disappear from the marrow, and the anemia may lessen or disappear without other therapy. Such a course has been observed, for example, in infectious diseases<sup>110,160</sup> or with the induction of remission in leukemia.<sup>170</sup> In other instances, improvement in the anemic state has been observed when folic acid, pyridoxine, or both were administered.<sup>139,156</sup> In general, folic acid is most likely to be effective when there is megaloblastosis and subnormal serum folate levels. Even under such circumstances, however, the response may be incomplete. In only a few patients have large doses of pyridoxine induced even modest increases in the hemoglobin level.<sup>139</sup> Sideroblastic anemia in myeloma may be a preleukemic change.<sup>144</sup>

It has been suggested that "secondary" sideroblastic anemia results from conditioned pyridoxine deficiency in a manner analogous to the development of folate deficiency in association with chronic hemolytic disease (page 579).<sup>156</sup> This hypothesis is supported by the relatively frequent association with evidence of folate lack. Also cited in support of this hypothesis is the high incidence of abnormalities in tryptophan metabolism<sup>156</sup>; however, these are more likely to be a manifestation of nonspecific induction of hepatic tryptophan pyrrolase than of pyridoxine deficiency.<sup>101</sup>

#### Sideroblastic Anemia Associated with Drugs and Toxins

Drugs and toxins that have been reported to cause sideroblastic anemia are listed in Table 18-4.

**Table 18-4. Drugs and Toxins Which May Produce Sideroblastic Anemia**

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1	Ethanol <sup>123,141</sup>
2	Drugs used in therapy of tuberculosis <sup>132,139,154,155,174</sup>
3	Lead <sup>104,127</sup>
4	Chloramphenicol <sup>103,139,157,140</sup>
5	Drugs used in therapy of neoplastic diseases nitrogen mustard <sup>109</sup> melphalan, <sup>144</sup> azathioprine <sup>118</sup>

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#### Alcoholism

Sideroblastic anemia occurs in about 30% of alcoholics who become ill enough to require hospitalization.<sup>123</sup> Blood hemoglobin ranges from 6 to 10 g/dl and pronounced dimorphism of circulating erythrocytes is observed.<sup>141</sup> The MCV is normal or increased, and the MCHC is normal or slightly reduced.<sup>123</sup> Transferrin saturation is increased (mean 80%) and 8 to 65% of the normoblasts are ringed sideroblasts, especially those in the polychromatophilic and orthochromic stages. Marrow hemosiderin stores are increased. These changes are almost always accompanied by vacuolization of the erythrocyte precursors<sup>150</sup> and by megaloblastic erythropoiesis, hypersegmentation of neutrophils, and reduced serum and red cell folate levels.<sup>141</sup> In controlled, sequential studies, the megaloblastosis preceded the sideroblastic changes.<sup>123</sup>

Withdrawal of alcohol is followed by a reticulocytosis that reaches a peak after five to 10 days, and by disappearance of ringed sideroblasts and an increase in blood hemoglobin levels.<sup>123,141</sup> Furthermore, the vacuolization and megaloblastosis of erythrocyte precursors disappear, and serum and red cell folate levels gradually increase.

Parenteral administration of pyridoxal-5-phosphate, the coenzyme form of vitamin B<sub>6</sub>, leads to remission, even if alcohol intake continues, but neither folate nor pyridoxine is effective under such circumstances.<sup>141</sup> This observation suggests that alcohol exerts an inhibitory effect on conversion of pyridoxine to the active coenzyme form.

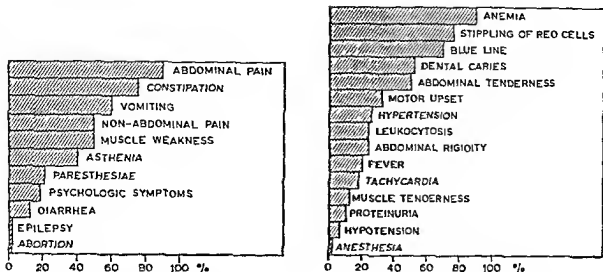


Fig. 18-10. Symptoms (left) and signs (right) in 50 cases of lead poisoning (From Dagg et al.<sup>120</sup> courtesy of the authors and Oxford University Press)

### Agents Used in Therapy of Tuberculosis

Sideroblastic anemia has been described in association with the treatment of tuberculosis with isonicotinic acid hydrazide (INH), pyrazinoic acid, and cycloserine, alone or in combination.<sup>132,134,135,174</sup> The number of reported cases is small in relation to the widespread use of these agents, suggesting that the complication is a rare one. In the reported cases, anemia was moderately severe (VPRC 0.20 to 0.26 l/l), and hypochromia was prominent on blood smear. Transferrin saturation was normal in some subjects, but increased in most. Ringed sideroblasts were invariably present in the bone marrow. Withdrawal of the offending drug led to reticulocytosis and gradual improvement in the VPRC. Alternatively, a similar response could be induced by administering large doses of pyridoxine while continuing the tuberculostatic drug.

It is probable that these drugs induce sideroblastic anemia by interfering with vitamin B<sub>6</sub> metabolism. INH reacts with pyridoxal to form a hydrazone, which is an inhibitor of pyridoxal kinase,<sup>153</sup> an enzyme required for the conversion of vitamin B<sub>6</sub> to its active coenzyme form. Cycloserine is an *in vitro* inhibitor of pyridoxal-dependent en-

zymes.<sup>112</sup> Serum pyridoxal concentrations were found to be subnormal in patients receiving INH.<sup>140</sup>

The relative rarity of the syndrome suggests that there may be additional unknown factors, perhaps genetic or nutritional, that render certain patients unusually susceptible to the antipyridoxine effects of the drugs.

### The Anemia of Lead Poisoning

Anemia is one of the most common manifestations of chronic lead poisoning (plumbism)<sup>120</sup> (Fig. 18-10). In experimental animals, lead administration induces an excess accumulation of iron within mitochondria of erythrocyte precursors.<sup>128,143</sup> In some human subjects with lead intoxication, ringed sideroblasts have been observed.<sup>104</sup> For these reasons, and also because the inhibitory effects of lead on heme synthesis have been well documented,<sup>100,127</sup> the anemia may be tentatively included among the sideroblastic anemias. Admittedly, however, a sizable series of patients has not yet been studied with respect to the incidence and degree of sideroblastic changes in the bone marrow.

Lead poisoning occurs in adults chiefly as the result of industrial exposure. In children with pica, intoxication may develop because of ingestion of lead-containing paint. The

**Table 18-5. Hematologic Changes in the Anemia of Lead Poisoning in Adults<sup>120,130</sup>**

	<i>Normal</i>	<i>Lead Poisoning</i>
Hemoglobin (g dl)	♂ 14-18 ♀ 12-16	10.7 (8-13)
VPRC (l/l)	♂ 0.40-0.54 ♀ 0.37-0.47	0.35 (0.29-0.43)
MCV (fl)	89 (83-96)	79 (70-92)
MCHC (g dl)	34 (32-36)	31 (27-36)
Reticulocytes (%)	1.6 (0.6-2.7)	4.4 (1.5-11.6)
Stippled cells (%)	rare	1.8 (0.1-7.5)
Leukocytes ( $\times 10^9/l$ )	4-11	4-15

principal manifestations are abdominal ("lead colic") and neurologic (Fig. 18-10), and the clinical picture bears some resemblance to that of acute intermittent porphyria (Chapter 42).<sup>120</sup>

The severity of the anemia ranges from mild to moderate and the red cells tend to be mildly hypochromic and microcytic (Table 18-5). In children, the morphologic changes are more pronounced than in adults, probably because of complicating iron deficiency.<sup>178</sup> The reticulocyte count usually is only slightly increased, but it may be greater in rare patients with overt hemolytic anemia.<sup>120</sup> Basophilic stippling may be prominent, but it is not always found nor does it correlate in degree with the intensity of lead exposure.<sup>130</sup> The stippling results from aggregation of ribosomes.<sup>100,141</sup> Osmotic fragility of the erythrocytes is decreased, and a further decrease occurs after 24 hours of sterile incubation.<sup>130</sup> Mechanical fragility is increased.<sup>130</sup> An electrophoretically "fast" hemoglobin similar to hemoglobin A<sub>3</sub> (page 172) may be found.<sup>115</sup> In about 15% of patients, an increased proportion of hemoglobins A<sub>2</sub> or F has been reported.<sup>100</sup> Erythroid hyperplasia is observed in bone marrow. Plasma iron levels tend to be normal or

slightly increased in adults,<sup>100,111</sup> and reduced in children, probably because the latter are deficient in iron.<sup>178</sup> Elevated plasma and urine lead levels (greater than 0.08 mg/24 hr) establish the diagnosis. When urinary lead excretion is borderline, the diagnostic accuracy may be improved by measuring urinary lead following an injection of 0.5 g calcium EDTA. Excretion of more than 0.8 mg of lead in 24 hours following the injection establishes the diagnosis.<sup>100</sup>

Studies of erythrokinetics in lead poisoning have demonstrated about a 20% decrease in mean red cell life span.<sup>101,111</sup> The pattern of destruction is random rather than senescent. Since the rate of <sup>51</sup>Cr elution from red cells is accelerated in lead poisoning, the rate of red cell destruction is overestimated with this isotope.<sup>101</sup> These effects probably result from damage to the red cell membrane.<sup>100</sup> Plasma iron turnover may be slightly decreased.<sup>104,111</sup> There is an increase in the "early labeled" bile pigment.<sup>104</sup> These kinetic findings suggest ineffective erythropoiesis accompanied by mild hemolysis.

Abnormalities in porphyrin metabolism<sup>127,130</sup> include greatly increased urinary delta-aminolevulinic acid (ALA) with normal levels of porphobilinogen (PBG). This combination of findings helps to distinguish plumbism from acute intermittent porphyria (Chapter 32), in which both PBG and ALA are excreted in excess. In plumbism, there is also an increase in urinary coproporphyrin and, to a lesser degree, urinary uroporphyrin. Free erythrocyte protoporphyrin is greatly increased. Stool porphyrins are normal. The abnormalities are presumed to result from the effects of lead on essential sulfhydryl groups of the heme biosynthetic enzymes. This presumption has been best established in the case of ALA dehydrase.<sup>116,149</sup> Heme synthetase probably is also affected.<sup>116,127</sup> The excessive urinary coproporphyrin excretion suggests that coproporphyrinogen oxidase activity may be inhibited by lead.<sup>116</sup>

Treatment of patients with lead poisoning requires the administration of chelating agents, especially calcium sodium ethylenediamine tetraacetic acid (EDTA). Since the lead chelate is also toxic, there may be a



Fig 18-11 Lead "line" in a patient with plumbism. Under a hand lens the lead line of blue-black lead sulfide, actually deposited in the gums immediately opposite to the teeth, appears as a clear row of dots or vertical streaks. (From Beiknap,<sup>101a</sup> courtesy of the author and the Journal of the American Medical Association.)

transient increase in encephalopathy and porphyrin excretion,<sup>116</sup> but this is less likely to occur if less than 0.5 to 1.0 g per day is given.<sup>100</sup> With EDTA treatment there is rapid urinary excretion of lead. Urine ALA, PBG, and coproporphyrin levels return to normal within several days, and the hemoglobin may reach normal in several weeks.<sup>104</sup> However, the damage to circulating red cells is not corrected, and the shortened life span may therefore continue until the defective cells are replaced with normal ones.

### Other Drugs

Sideroblastic anemia has been reported in several patients given chloramphenicol in total doses of 17 to 56 g.<sup>103,129,139,140</sup> The abnormalities disappeared when the drug was withdrawn. Chloramphenicol may act by inhibiting the synthesis of mitochondrial proteins, including heme synthetase.<sup>157</sup> There is no demonstrated connection between this syndrome and chloramphenicol-induced aplastic anemia (page 1750).

Transient sideroblastic changes have been observed as a response to antineoplastic agents, such as nitrogen mustard<sup>109</sup> and azathioprine.<sup>118</sup> Similarly, sideroblastic anemia

has been observed as a complication in patients with myeloma treated with melphalan.<sup>141</sup> In this latter group, leukemia often developed within several months.

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# The Normocytic, Normochromic Anemias

### Classification and Diagnostic Approach

Acute Posthemorrhagic Anemia

Congenital Dyserythropoietic Anemias

The Anemia of Chronic Renal Insufficiency

Anemia in Cirrhosis and Other Liver Dis-

orders

Anemias Associated with Endocrine Dis-

orders

THE normocytic, normochromic anemias are those in which the MCV and MCHC are within normal limits or, at least, do not deviate greatly from normal. One problem in approaching and classifying the anemias that fall into this category is that many of them have variable morphologic characteristics. Thus, for example, the anemias associated with myxedema, liver disease, or acute hemorrhage may be either normocytic or slightly macrocytic. These anemias have been included in this chapter because their most common or least complicated presentation is that of a normocytic normochromic anemia. On the other hand, the anemia of chronic disorders, although most often normocytic and normochromic, is more conveniently discussed with the hypochromic microcytic anemias because the pathogenesis of the disorder and the tests used in its detection and diagnosis are best understood in that context (Chapters 16 and 18). In still other disorders, such as iron deficiency, the typical morphologic features become apparent only when

the condition is fully developed, and early in the course the anemia may be normocytic and normochromic.

Another problem that complicates understanding of the normocytic normochromic anemias is that they, in contrast to the macrocytic anemias (Chapter 14) or the hypochromic, microcytic anemias (Chapter 16), are not clearly related to one another by common pathogenetic mechanisms. Instead, they constitute a group of remarkably diverse etiologic background. In many instances the anemia is of only incidental importance, ie, a minor manifestation of a systemic disease with other, more serious consequences. Sometimes, however, it is the first evidence of the disease and the sign leading to investigations resulting in the discovery of the underlying disorder.

## Classification and Diagnostic Approach

Despite the varied etiologic background and the often incidental nature of the normocytic anemias, it is possible to classify them in a way that forms a basis for diagnostic investigation (Table 19-1).

As a first step, the physician should determine whether the marrow response is appropriate to the degree of anemia. When bone marrow function is unimpaired, erythropoiesis can increase six- to eight-fold and the M:E ratio becomes reduced because of ac-

**Table 19-1. Classification of the Normocytic, Normochromic Anemias**

- 
- A Anemia associated with appropriately increased erythrocyte production
    - 1 Posthemorrhagic anemia
    - 2 Hemolytic anemia (Chapter 20)
  - B Anemia with impaired marrow response
    - 1 *Intrinsic bone marrow disease*
      - a Hypoplasia (Chapter 56)
        - 1 Hypoplastic or aplastic anemia
        - 2 Erythroblastic hypoplasia
      - b Disorders characterized by infiltration of the bone marrow
        - 1 Leukemia (Chapters 47-49)
        - 2 Myeloma (Chapter 52)
        - 3 Other myelophthisic anemias (Chapter 57)
      - c Dyserythropoietic anemias
    - 2 Decreased erythropoietin secretion
      - a Impaired source
        - 1 Renal anemia of renal insufficiency
        - 2 Hepatic (?) anemia of liver disease
      - b Reduced stimulus (decreased tissue oxygen needs) anemia of endocrine deficiency
      - c Protein-calorie malnutrition (Chapter 4)
      - d Anemia of chronic disorders (Chapter 18)
    - 3 Deficiency or unavailability of iron (early normocytic, normochromic, later hypochromic microcytic)
      - a Iron deficiency (Chapter 17)
      - b Anemia of chronic disorders (Chapter 18)
- 

celerated proliferation of erythroid elements. Fortunately, it is rarely necessary to resort to marrow examination to evaluate marrow response. The most useful signs are found in the blood, where *reticulocytosis* is prominent and, on routinely stained smears, polychromatophilic macrocytes are detected. Ferrokinetic studies, also rarely necessary, disclose increased rates of plasma and erythroid iron turnover (Table 19-2, Hemolytic Anemia). These manifestations of appropriate marrow response are typical of hemolytic anemia (Chapter 20) and, to a somewhat lesser extent, posthemorrhagic anemia (page 695). The history, physical examination, and the signs of excessive erythrocyte destruction, such as hyperbilirubinemia and excessive urobilinogen excretion, easily differentiate these two conditions.

When anemia is found and the erythropoietic response described above is lacking, it may be presumed that the underlying disorder directly or indirectly affects the bone marrow. Suspicion of *intrinsic marrow disease* should be particularly great when there is

associated leukopenia and thrombocytopenia, or when morphologic abnormalities suggestive of marrow infiltration are found on the blood smear. These include nucleated red cells, "tear-drop" poikilocytes, immature leukocytes, and large, bizarre platelets or megakaryocyte fragments. With either type of change, marrow aspiration and biopsy should be performed. Hypoplasia is easily detected by this procedure, and infiltrating elements may be demonstrated. The dyserythropoietic anemias may be identified by the presence of prominent multinuclearity of the marrow normoblasts.

When no intrinsic disease is apparent on study of the marrow, an *indirect effect on erythrocyte production* should be considered. Several disorders are associated with reduced secretion of the erythropoietic hormone, erythropoietin (Table 19-1). Erythropoietin assays are not generally available, but fortunately it is not necessary for diagnostic purposes to establish that erythropoietin levels are low in these conditions, because screening tests usually will uncover an underlying sys-

temic disease (Table 19-1, B2). For this purpose, the functions of the kidneys, liver, and thyroid gland should be assessed by means of appropriate biochemical tests. Although severe protein-caloric malnutrition is accompanied by reduced erythropoietin secretion and a mild normocytic anemia (Chapter 4), such undernutrition is rare in temperate countries and unlikely to be confused with other systemic disorders. Finally, it is helpful to study the serum iron and iron-binding capacity, for these, in conjunction with marrow iron stains, should make it possible to detect either the anemia of chronic disorders or early iron deficiency (Chapter 16). This diagnostic approach is outlined in Figure 19-1.

## Acute Posthemorrhagic Anemia

The anemia caused by blood loss occurs in association with a large variety of underlying diseases. Bleeding may be obvious as, for example, when profuse hemorrhage occurs from a body orifice or from an external wound. Alternatively, when bleeding occurs

within the gastrointestinal body cavity or tissue space, the problem may not be immediately apparent and may present a diagnostic challenge. Certain causes of hemorrhage are unique to the newborn; these have been discussed in Chapter 13 (page 560) and are listed in Table 13-1.

When the blood loss occurs in small amounts over a prolonged period of time, no anemia develops until iron stores have been depleted. In such circumstances, the hematologic findings are those of iron-deficiency anemia (Chapter 17). On the other hand, when larger amounts of blood are lost, anemia may develop even though iron stores remain adequate. This latter type is designated *acute posthemorrhagic anemia*.

### Clinical Description

The manifestations of hemorrhage depend on the rate and magnitude of the bleeding, the time elapsed since it took place, and whether it is external or occult. In addition, the presence of complicating diseases and the cardiovascular status of the patient, as well as his age, nutritional adequacy, and emo-

Table 19-2. Ferrokinetic Measurements in Certain Normocytic Anemias<sup>58</sup>

Measurement	Normal	Hemolytic Anemia*	Myxedema	Renal Disease		Liver Disease	
				Moderate†	Severe†	Kimber et al <sup>116</sup>	Boivin et al <sup>103</sup>
Plasma iron ( $\mu\text{g/dl ml}$ )	105	127	62	76	220	87	134
Iron clearance $t_{1/2}$ (minutes)	88	24	84	79	221	96	64
Plasma iron transport (mg/day/dl ml)	0.7	3.4	0.5	0.8	0.8	0.7	1.6
Red cell iron utilization (%)	80	57	79	81	34	65	87
Erythrocyte iron turnover (mg/day/dl)	0.6	1.9	0.4	0.7	0.2	0.5	1.4

\*Hereditary spherocytosis

†As judged by transfusion requirement: moderate, less than 400 ml/month; severe, greater than 400 ml/month

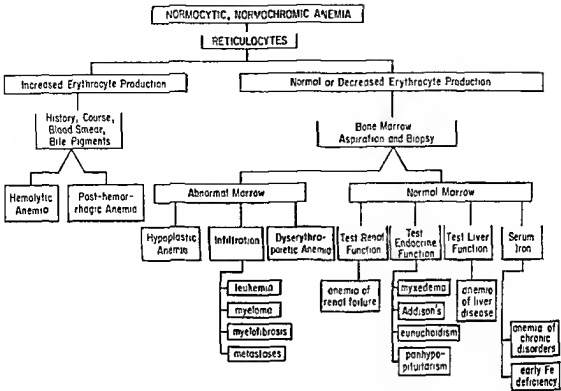


Fig 19-1 Diagrammatic representation of the diagnostic approach to normocytic normochromic anemia. The closed boxes represent findings, the open boxes procedures.

tional stability, may modify the response to bleeding

Following a single acute hemorrhage of brief duration, a characteristic sequence of events is observed, which may be divided into two phases. The first phase, lasting about one to three days, is dominated by manifestations of hypovolemia, and there may be little or no anemia. The second phase, which follows restoration of the blood volume to near normal levels, is characterized by anemia and signs of active erythrocyte regeneration. When the hemorrhage is prolonged or recurrent, manifestations of hypovolemia and those of regenerative anemia may occur at the same time, or may wax and wane.

Most young, healthy subjects can tolerate the rapid loss of 500 to 1000 ml of blood (10 to 20% of the blood volume) with few, if any symptoms.<sup>1</sup> However, approximately 5% of the population experience a "vasovagal reaction" to blood loss of this magnitude.<sup>2,10</sup>

The reaction consists of weakness, sweating, nausea, and a fall in heart rate and blood pressure, often followed by lightheadedness and loss of consciousness (syncope or fainting). Whether these subjects react as they do because of physiologic or psychic differences in their makeup is unknown; however, they exhibit two dominant personality traits: hypochondriasis and a tendency to react to stress with hopelessness, depression, or despair.<sup>10</sup>

With the rapid loss of 1000 to 1500 ml of blood (20 to 30% of the blood volume), previously healthy subjects remain asymptomatic while at rest in a recumbent position but may experience lightheadedness and hypotension when upright. They also respond to exertion with an unusual degree of tachycardia.<sup>3</sup>

When 1500 to 2000 ml (30 to 40% of the blood volume) is lost, symptoms develop even when the subject is recumbent. Thirst,

shortness of breath, clouding or loss of consciousness, and sweating appear. The blood pressure, cardiac output, and central venous pressure are reduced.<sup>6,13</sup> The pulse usually becomes rapid and low in volume ("thin and thready"); however, in some patients, possibly in the "reactors" described above, bradycardia is observed. A redistribution of blood flow occurs as the result of adrenergic stimulation; flow to the heart, lungs, liver, and brain are favored at the expense of the skin, muscles, kidneys, and gastrointestinal tract. As a result of these hemodynamic changes, the skin, especially that of the extremities, becomes cold, clammy, and pale. Urine volume becomes reduced, and salt and water are conserved because of decreased renal blood flow and the actions of aldosterone and antidiuretic hormone.

When rapid blood loss exceeds 2000 to 2500 ml (40 to 50% of the blood volume), a severe state of shock ensues. Lactate acidosis resulting from inadequate tissue perfusion may be observed. At this stage, there is a significant risk that the shock will become irreversible, leading to death.

### Hematologic Findings

Immediately following hemorrhage, plasma volume and red cell mass are reduced in proportional amounts; consequently, there is no decrease in the VPRC (Fig. 19-2).<sup>1,3</sup> Over the next several days, the blood volume is restored by an influx of fluid containing electrolytes and protein. In recumbent subjects, most of the expansion of plasma volume occurs within the first 24 hours and is accounted for chiefly by movements of water and electrolytes. In ambulatory subjects, the plasma volume expansion is accomplished more slowly and is related primarily to mobilization of the extravascular albumin pool.<sup>1</sup> Because of the time required for these alterations in blood volume, the amount of blood loss tends to be underestimated by the degree of anemia, especially early in the course. The VPRC may not reach the minimum value until three or more days after the hemorrhage ceases (Fig. 19-2).

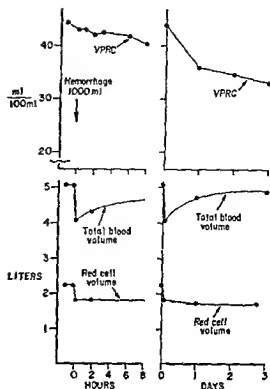


Fig. 19-2. The sequence of events during the first eight hours (left) and the first three days (right) following a single hemorrhage of 1.0 liter in a normal adult. The anemia develops slowly, and the VPRC does not reach the lowest values until about 72 hours after the hemorrhage. (Data from Ebert et al.<sup>13</sup> Figure from GR Lee<sup>14</sup> in *Text-Practice of Medicine*, Harper & Row, by permission.)

When first detected the anemia is normocytic and normochromic and there are few signs of erythrocyte regeneration. However, erythropoietin secretion is stimulated, and hyperplasia of marrow erythroid elements begins. This change may be evident on marrow examination within three to five days after hemorrhage.<sup>5</sup> Some increase in reticulocytes is usually perceptible within three to five days, and maximum values are reached at six to 11 days.<sup>11</sup> The degree of reticulocytosis is related to the magnitude of hemorrhage, but rarely exceeds 15%. The reticulocyte index (Chapter 20, page 731) may reach levels as high as 5.0 at 10 days.<sup>5</sup> Other signs of erythrocyte regeneration include polychromatophilia and macrocytosis, and the MCV may become transiently increased (Fig. 19-3). If the patient is seen for the first time during this stage, the findings may be mis-

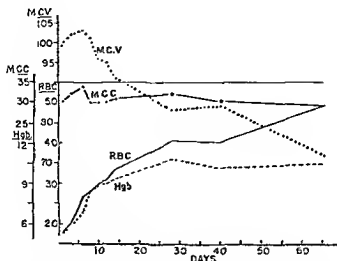


Fig 19-3. Temporary increase in mean corpuscular volume associated with acute blood loss

taken for those of hemolytic anemia; however, signs of increased bilirubin production are conspicuously absent, unless bleeding has occurred into a body cavity or tissue space.

During or immediately after hemorrhage, the platelet count, the whole blood coagulation time, and the plasma fibrinogen level may be reduced.<sup>10</sup> These abnormalities usually are transient and their values return to normal within 15 minutes after bleeding ceases. Thereafter, the platelet count increases, often reaching levels above normal within an hour. Values as great as  $1000 \times 10^9/l$  may be observed. If severe shock develops, disseminated intravascular coagulation (Chapter 38) may occur.

Neutrophilic leukocytosis often follows hemorrhage, the leukocyte count becoming maximal at two to five hours. Typically, the leukocyte count is 10 to  $20 \times 10^9/l$  but counts as high as  $35 \times 10^9/l$  have been observed. The leukocytosis is explained by the effect of epinephrine on the mobilization of granulocytes from the marginal pool (Chapter 6) and by their release from the marrow granulocyte reserve.

## Diagnosis

The sudden onset of severe anemia, when not due to an obvious cause, should direct

suspicion to the gastrointestinal tract or, if the patient is a female, to the reproductive organs as sites of hemorrhage. Acute hemolytic anemia is much less common and is accompanied by hyperbilirubinemia, hemoglobinemia, and other signs (Chapter 20). As already mentioned, hemorrhage into one of the body cavities, or into a cyst, may be accompanied by hyperbilirubinemia arising from the breakdown and absorption of the blood.

## Management

During the hypovolemic phase, therapy should be directed toward restoration of the blood volume. When the situation is urgent, this can be accomplished with intravenous infusions of saline, albumin, plasma, or dextran solutions.<sup>7,8,9,12</sup> Whole blood represents the replacement therapy of choice,<sup>14</sup> and, if large amounts (more than 4 units per day) are required, fresh blood should be used. The risks and precautions related to transfusion therapy have been discussed in Chapter 11. Response to therapy must be gauged by observations of the pulse rate and blood pressure and the change in these parameters when the upright position is assumed. For reasons already discussed, the VPRC is of limited value as a guide to therapy. In critically ill

subjects and in complex clinical circumstances, it is often necessary to insert a catheter into a central vein or the pulmonary artery so that changes in pressure volume can be monitored as volume is replaced.

Once the emergency has been dealt with, remedial measures must be directed at the bleeding lesion, according to the nature of the underlying disease.

The anemia itself rarely requires specific therapy. If iron stores are adequate, the marrow is capable of regenerating the lost red cells and the rate of regeneration is not accelerated by administration of iron, folate, vitamin B<sub>12</sub>, or other "hematinic" substances. Schipdt found that, no matter how great the blood loss had been, the rate of erythrocyte regeneration was such that a level of about  $4.5 \times 10^{12}$  red cells per l was reached in about 33 days, the curve of regeneration rising more sharply in subjects having the more severe cases of anemia.<sup>11</sup> The red cell count was usually restored entirely to normal in four to six weeks, although the hemoglobin lagged behind, reaching normal only after six to eight weeks. In blood donors from whom an average of 555 ml of blood had been removed, an average drop in hemoglobin of 2.3 g/dl was observed, an amount which required 50 days to replace.<sup>4</sup>

After 10 to 14 days there should be no morphologic evidence of active red cell regeneration. The leukocyte count should be normal after three or four days. Continued bleeding is suggested by a maintained high level of reticulocytes. Persistent leukocytosis may result from the same cause or may be due to hemorrhage into body cavities or to complications. The latter, particularly infections, tend to delay the hematopoietic response.

## Congenital Dyserythropoietic Anemias (CDA)

Congenital dyserythropoietic anemias are rare familial disorders characterized by the association of refractory anemia with multinuclearity, karyorrhexis, and other bizarre nuclear abnormalities of erythrocyte precursors

in the bone marrow. Three types of CDA have been distinguished on morphologic grounds.<sup>27</sup> Type I is characterized by binuclearity, intranuclear chromatin bridges, and by changes in nuclear chromatin structure similar to those found in megaloblasts; type II, by normoblastic chromatin, bi- and multinuclearity and pluripolar mitosis; and type III, by pronounced multinuclearity (up to 12 nuclei per cell) in enormous (up to 50 to 60  $\mu$ m diameter) erythrocyte precursors ("gigantoblasts").

The degree of anemia is extremely variable. In some patients a thalassemia-like picture, evident in infancy, has been described. However, in about half the reported cases, the anemia has been of moderate degree, so that discovery was delayed until early adulthood.<sup>22</sup> In all three types, the morphologic and kinetic criteria of ineffective erythropoiesis have been present. Erythroid hyperplasia of the marrow is pronounced, but the reticulocyte count is not increased. Plasma iron transport is markedly increased, but red cell utilization is reduced. Often, indirect hyperbilirubinemia (as great as 3.5 to 4.0 mg/dl) and increased fecal urobilinogen excretion have been noted. Nevertheless, red cell survival has been normal or only slightly reduced. Endogenous carbon monoxide production has exceeded that which could be accounted for on the basis of destruction of circulating cells. Secondary hemochromatosis has been a common complication. The abnormal proliferation affects the erythrocyte line only; leukocytes, platelets, and their precursors remain normal.

### Type I

This is probably the rarest of the three types, having been reported in only six patients, including two pairs of siblings.<sup>28</sup> The anemia was mildly macrocytic, and anisocytosis and poikilocytosis were prominent. Binucleated cells, cells with incompletely separated or multilobulated nuclei, megaloblastic erythrocyte proliferation, and erythrophagocytosis were common findings. Additional morphologic abnormalities on electron

microscopy included uneven condensation and a spongy appearance of nuclear chromatin, loss of nuclear envelope, chromatin bridges between the nuclei of divided cells, and persistence of cytoplasmic microtubules.<sup>28</sup>

### Type II

This variety of CDA is also known as HEMPAS, an acronym derived from the initials of Hereditary Erythroblast Multi-nuclearity with Positive Acidified Serum test.<sup>23</sup> It has been reported in about 40 individuals and appears to be inherited as an autosomal recessive trait.<sup>22,23,21,29</sup> The principal clinical findings are normocytic anemia, variable jaundice, and hepatosplenomegaly. Anisocytosis and poikilocytosis are prominent. Multiple nucleoli are found in 10 to 40% of marrow normoblasts, especially those in the later stages of maturation. These cells appear to synthesize DNA at a markedly reduced rate.<sup>31</sup> However, in contrast to type I cases, no megaloblastic features are found. Phagocytosis of erythrocytes and normoblasts by reticulum cells, and Gaucher-like histiocytes have been observed in some patients.<sup>24</sup>

The erythrocytes resemble those in PNH (Chapter 29) in that they are unusually susceptible to hemolysis in most acidified normal sera and by anti-i and anti-I antibodies.<sup>23</sup> However, acid hemolysis does not occur in the patient's own serum. They also differ from PNH erythrocytes in that they do not hemolyze in the sugar-water test. These abnormalities imply a membrane defect, as yet undefined.<sup>23</sup> In one case studied by electron microscopy, the cytoplasmic membranes appeared to be doubled.<sup>31</sup> Additional red cell abnormalities include a pronounced increase in the activity of phosphoglucose isomerase, triosephosphate isomerase, and certain other glycolytic enzymes.<sup>29</sup>

### Type III

This was probably the first variety of CDA to be described.<sup>20</sup> It has been reported in

about 23 subjects in four families,<sup>22,26</sup> and the pattern of inheritance suggests autosomal dominant transmission.<sup>23</sup> The anemia has been normocytic or slightly macrocytic; a few very large erythrocytes have been seen on blood smear. As many as 30% of the erythrocyte precursors have been multinucleated. Remarkable variability in the amount of DNA per normoblast nucleus has been observed.<sup>26</sup> In one family, an abnormal hemoglobin was detected chromatographically in both affected and apparently healthy subjects.<sup>21</sup> In another patient, the erythrocytes were more readily agglutinated by anti-I antibodies than normal, but the reaction to the acidified serum test was negative.<sup>26</sup>

## The Anemia of Chronic Renal Insufficiency

Anemia accompanies chronic renal insufficiency with such regularity that it is as typical of the disorder as azotemia.<sup>52</sup> In the complex clinical settings associated with uremia, anemia may occur for many reasons. If the kidneys are infected or inflamed, the anemia of chronic disorders (Chapter 18) is likely to be observed. Iron-deficiency anemia (Chapter 17) may develop because of blood loss from the genitourinary or gastrointestinal tracts or into the hemodialysis apparatus.<sup>42,47,78</sup> Megaloblastic anemia due to folate deficiency also may occur in patients receiving periodic dialysis.<sup>62</sup> In certain types of renal disease, including the hemolytic-uremic syndrome and malignant hypertension, microangiopathic hemolytic anemia may be found (Chapter 28).

In addition, anemia occurs in uremia even in the absence of any of the above mechanisms. Failure of both the excretory and the erythropoietin-secreting functions of the kidney is implicated in its pathogenesis. Although red cell survival may be moderately shortened, the predominant kinetic characteristic is reduced red cell production. It is this "hypoproliferative"<sup>42</sup> anemia that is usually meant by the phrase "anemia of chronic renal insufficiency."



### Clinical Description

In most instances, the patient seeks medical attention because of symptoms related to the underlying renal disease, and anemia is an incidental finding. Occasionally, however, the renal symptoms are so subtle and slowly progressive that the patient's chief complaints are of pallor, exertional dyspnea, or other signs of the cardiovascular adjustment to anemia. When appropriate tests of renal function have not been performed, hematologic consultation may be requested for the diagnosis of what appears to be a mysterious, normocytic normochromic anemia.

A very rough correlation has been observed between the degree of anemia and the degree of renal impairment, as judged by the blood urea nitrogen (BUN) or creatinine level<sup>53,55,67,93</sup> (Fig 19-4). A significant hyperbolic correlation also was observed between glomerular filtration rate (GFR) and the blood hemoglobin level.<sup>75</sup> Anemia developed only when GFR was reduced to less than 30% of normal, a level corresponding to an increase in plasma creatinine to about

2 to 4 mg/dl. Pronounced anemia was observed when the GFR fell below 20 ml/minute/1.73 m<sup>2</sup>. The nature of the renal disease leading to uremia has little effect on the development of anemia; however, the degree of anemia may be somewhat less in patients with hypertension<sup>64</sup> and considerably less in patients with polycystic disease.<sup>49</sup> In the Salt Lake Veterans Hospital Hemodialysis Unit, the mean VPRC in nine subjects with polycystic disease was 0.37 l/l as compared with 0.23 l/l in 27 subjects with other types of chronic renal failure.<sup>95</sup>

### Laboratory Findings

The anemia may progress as renal failure worsens, but ultimately in most patients the VPRC becomes stabilized between 0.15 and 0.30 l/l and does not fall to lower levels unless complications develop.<sup>42</sup> Since regulation of body water and electrolyte balance is impaired in renal disease, both hydremia and dehydration are common. Thus, the apparent degree of anemia may be exaggerated or minimized by alterations in plasma volume.

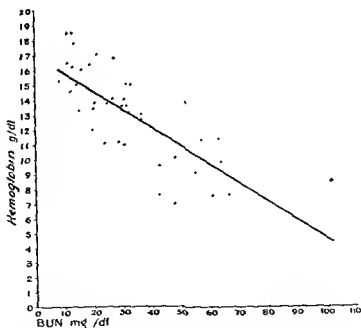


Fig 19-4. Relation between blood urea nitrogen (BUN) and blood hemoglobin concentration (From Kaye,<sup>67</sup> courtesy of author and Journal of Laboratory and Clinical Medicine)

The erythrocytes usually are normocytic and normochromic,<sup>46, 53, 67, 70, 86</sup> but slight macrocytosis occasionally has been observed.<sup>70</sup> Often the red cells appear normal or nearly so on blood smear; characteristically, little variation in size or shape is seen, and cells that are polychromatophilic or stippled are unusual. Occasionally, however, "burr" cells (Chapter 13) may be observed along with some triangular, helmet-shaped or fragmented cells. The reticulocyte count often is within normal limits,<sup>46</sup> but may be moderately increased.<sup>53, 68, 70</sup> In one study, reticulocytes were normal when the BUN was less than 130 mg/dl, but, at higher BUN levels, their number often was increased.<sup>89</sup> The highest values (average 6%) were observed when the BUN was between 300 and 350 mg/dl.

The leukocyte count often is normal, but slight neutrophilic leukocytosis may be observed. In one series, the leukocyte count averaged  $10.7 \times 10^9/l$ .<sup>46</sup> The platelet count was either normal or slightly increased.<sup>46</sup> However, platelet function may be severely impaired, resulting in defective hemostasis<sup>94, 90</sup> (Chapter 35).

The bone marrow tends to be moderately hypercellular, and erythroid hyperplasia may be observed. The average M:E ratio was found to be 2.5:1 in one study.<sup>46</sup> Erythrocyte maturation appeared morphologically normal. In some instances, especially when the renal failure is relatively acute, hypoplasia of erythroid elements may be found.<sup>78, 85, 88</sup>

Measures of bile pigment production tend to give normal or somewhat increased values. The serum bilirubin was within normal limits in all of the 26 patients in one series.<sup>70</sup> However, the hemolytic index, a measure of urobilinogen excretion in relation to total circulating hemoglobin (Chapter 5, page 217), was increased in eight of 21 patients in two studies.<sup>67, 70</sup> In only three of these patients did the hemolytic index exceed 40 (normal, 11 to 21).

Values for serum iron vary considerably in renal disease.<sup>53, 70</sup> As a general rule, the value is normal when renal impairment is mild. With more severe disease, some investigators

have observed a fall in serum iron levels,<sup>67</sup> whereas others have found hyperferremia.<sup>53, 58</sup> This lack of agreement may be partially explained by complicating factors. For example, if iron overload has been induced by multiple transfusions, hyperferremia is to be expected.<sup>54</sup> On the other hand, if the renal disease is complicated by blood loss or by inflammation or infection, hypoferremia results. Whether there is a characteristic disturbance of serum iron concentration in "uncomplicated" uremia remains to be determined. One group of investigators found gastrointestinal absorption of iron, measured with the total body counter, to be reduced in patients with chronic renal failure.<sup>43</sup> Others, using the less satisfactory double isotope method, found iron absorption to be related to disturbances in iron balance and to be unrelated to degree of anemia, rate of erythropoiesis, or degree of azotemia.<sup>87</sup>

Free erythrocyte protoporphyrin may be normal or moderately increased.<sup>70</sup> Erythrocyte lactate dehydrogenase (LDH) is within normal limits.<sup>71</sup>

### Kinetic Studies and Pathogenesis

The major pathogenetic factor in the anemia of uremia is failure of the bone marrow to produce adequate numbers of red blood cells. When anemia is severe, ferrokinetic studies typically demonstrate a normal plasma iron transport rate, but red cell iron utilization and erythrocyte iron turnover are reduced (Table 19-2).<sup>42, 70, 71</sup> With milder degrees of anemia, ferrokinetic measurements tend to be near normal (Table 19-2).

It must be emphasized, however, that in anemic patients such "normal" values indicate an insufficient marrow response to the stimulus of anemia.

The defect in erythropoiesis is at least partially explained by failure of the renal erythropoietin-secreting mechanism (Chapter 4, page 180). In the great majority of patients with renal disease, plasma erythropoietin is not detectable even when anemia is severe.<sup>45, 56, 79, 82</sup> It is probable that, in man, extrarenal sources of erythropoietin<sup>81, 83</sup> ac-

count for the maintenance of a steady, but subnormal rate of red cell production in uremic and anephric subjects. The extrarenal erythropoietin secretion does not, however, appear to increase sufficiently in response to anemia to compensate for deficiencies at the renal source.

In addition to erythropoietin deficiency, other factors may depress erythropoiesis in patients with uremia. Evidence has been presented for the presence of an erythropoietin-inhibiting substance, possibly of renal origin, in the plasma of some patients with chronic renal failure.<sup>59,79</sup> Moreover, the uremic state appears to inhibit the response to exogenously administered erythropoietin.<sup>44,92</sup> Finally, the rate of erythropoiesis has been found to improve in patients treated with dialysis, even though plasma erythropoietin levels have remained unchanged.<sup>56,68,72</sup>

A second pathogenetic factor is erythrocyte survival, which, although often within normal limits, may be slightly to moderately reduced. In only 20 of 106 patients studied with the <sup>51</sup>Cr technique by five different groups of investigators<sup>52,67,68,89,93</sup> was the erythrocyte life span clearly reduced. As determined by the Ashby method, erythrocyte half-survival time averaged 44 days and ranged from 21 to 68 days in 10 uremic patients (normal 52 to 68 days).<sup>70</sup> When measured with DF<sup>32</sup>P, erythrocyte survival averaged 66 days and ranged from 30 to 100 (normal 120 days) in 13 patients whose azotemia was controlled by dialysis.<sup>56</sup> The degree to which red cell survival is shortened has been alleged to be related to the degree of azotemia,<sup>42</sup> but others have found this relationship to be unimpressive.<sup>55</sup>

Cross-transfusion studies usually have indicated that the hemolytic factor, when present, is extracorporeal. Survival of normal cells transfused into patients was shortened, whereas the patient's cells survived normally in normal recipients.<sup>52,67,70</sup> Less commonly, shortened survival of the patient's cells has been observed even in normal subjects.<sup>67</sup> In some patients, splenic sequestration of red cells may be prominent.<sup>63</sup>

The mechanisms of increased erythrocyte

destruction have not been clearly delineated. The reaction to the Coombs' test is negative.<sup>70</sup> Erythrocyte osmotic fragility usually is within normal limits.<sup>52,70</sup> Mechanical fragility is normal<sup>70</sup> or slightly increased.<sup>52</sup> The autohemolysis test has been found to give normal values by some<sup>52</sup> and increased values by others.<sup>60</sup>

A number of potentially toxic substances accumulate in uremia, and it is conceivable that one or more of them may interfere with erythrocyte function, resulting in premature cell death. As yet, however, neither a specific toxin nor impairment of a single metabolic function has been clearly implicated in the reduced red cell survival. Welt and his co-workers found that red cell sodium concentration was increased in about 25% of patients with uremia.<sup>94</sup> In the patients with this abnormality, erythrocyte ATPase activity and active sodium efflux were found to be reduced. These observations suggested that a defect in membrane ion transport might explain reduced red cell survival. In contrast, however, Lichtman and Miller, in 20 uremic subjects, could detect no impairment of ATPase activity or of ATP utilization.<sup>69</sup>

Evidence of impaired erythrocyte glycolysis in uremia has been reported<sup>71</sup>; however, most studies have found the overall glycolytic rate to be increased,<sup>69,83</sup> probably because of the elevation in plasma inorganic phosphorus.<sup>69</sup> The increase in glycolytic rate probably accounts for the observed increase in red cell ATP levels.<sup>69</sup> Hemoglobin oxygen affinity is reduced,<sup>76</sup> presumably because of increased erythrocyte ATP and 2,3 DPG levels. Such abnormalities would not be expected to cause reduced red cell survival.

Evidence has also been presented that shortened red cell survival in some uremic patients results from impaired function of the hexosemonophosphate (HMP) shunt. This abnormality was induced by accumulation of a toxic, nondialyzable substance, possibly chloramine derived from chlorinated tap water use in dialysis media.<sup>66</sup> In these studies, shortened erythrocyte survival correlated well with the results of the ascorbate-cyanide screening test (page 736), which was abnor-

mal in 43 of 100 uremic patients. These patients developed severe, Heinz body hemolytic anemia if given primaquine, and Heinz bodies were also observed in splenectomized, nephrectomized patients not given this drug. Thus, the hemolytic pattern in these patients resembled that observed in G-6-PD deficiency (Chapter 23). Such an HMP shunt abnormality also may explain previously observed abnormalities in red cell glutathione content and stability in uremia.<sup>91</sup>

# Management and Course

In patients with mild renal failure and few symptoms, no therapy is required for the anemia. Eventually most patients with progressive disease require one of two major therapeutic regimens, namely, regular maintenance hemodialysis or renal homotransplantation. These procedures have completely changed the prognosis in uremia. The management and response of the anemia differ, depending on which of these two modes of therapy is employed.

Although maintenance hemodialysis<sup>13,47,48,49,56,61,68,78,96</sup> is an effective means of controlling most of the symptoms and biochemical manifestations of renal failure, anemia continues to be a problem in many patients on this regimen. In such patients, it appears preferable to give no blood transfusions unless symptoms due to anemia or blood loss occur.<sup>48,49,61,95</sup> Usually the VPRC stabilizes at a level above 0.15 l/l, and most patients tolerate this degree of anemia remarkably well. Even lower levels have been tolerated by young patients who have no associated disorders.<sup>78</sup> After one to 10 months or more of treatment, modest reduction in the degree of anemia or in transfusion requirements has been observed.<sup>48,56,61,78</sup> In one study of 36 patients, the VPRC rose from an average of 0.21 l/l (range 0.12 to 0.29) to 0.27 (range 0.16 to 0.48) on hemodialysis.<sup>48</sup> The improvement results chiefly from an increase in red cell production, even though erythropoietin levels do not increase.<sup>56,68,72</sup> The use of routine transfusion to maintain the VPRC at levels around 0.25 l/l has been

considerably less satisfactory. With such a regimen, an average of 3.1 units of blood per month (range 0.5 to 5.0 units/month) was required<sup>91</sup> as compared with 0.34 units per month<sup>19</sup> or less<sup>96</sup> when given only after symptoms developed. Not only is the risk of hepatitis and iron overload<sup>94</sup> significantly increased by routine transfusion, but erythropoiesis is depressed.<sup>61,82</sup>

When transfusions are withheld, iron deficiency is a common complication of hemodialysis, affecting perhaps half or more of the patients.<sup>47,78,96</sup> Loss of blood in the dialysis apparatus is the usual cause.<sup>48,49,78</sup> Detection of iron deficiency may be difficult in this situation because the usual morphologic criteria may be absent<sup>96</sup> and some<sup>49</sup> but not all<sup>47</sup> investigators have found even the plasma iron levels to be misleading. Various approaches to the treatment and prevention of the iron deficiency have been employed. It seems reasonable to administer oral iron prophylactically to all hemodialysis patients. The dose should be increased to tolerance, eg, as much as 600 mg of elemental iron per day (3 ferrous sulfate tablets three times daily) because of possibly defective intestinal absorption.<sup>43</sup> Parenteral iron also has been used, eg, 100 mg of iron as Imferon given intravenously after each dialysis.<sup>47,48,78</sup> This procedure effectively relieves the iron deficiency, but if continued indefinitely results in significant hepatic iron overload.<sup>49</sup> Alternatively, single or multiple injections totaling 1.0 g of iron have been given only to patients with clearly subnormal plasma iron values.<sup>78,96</sup> In 10 patients so treated, hemoglobin increases of 2.4 g/dl were observed over a four- to six-week period.<sup>96</sup>

Androgens may be useful adjuncts to therapy in patients who continue to develop symptoms of anemia and to require blood transfusion even when iron stores are adequate.<sup>50,87</sup> Testosterone enanthate, given intramuscularly, has been used in doses of 400 to 600 mg/week. A mean increase in VPRC of 0.056 l/l was observed with this treatment,<sup>87</sup> eliminating the need for transfusion.<sup>50,87</sup> Androgens also appear to increase erythrocyte 2,3 DPG in patients with

renal disease, a change which facilitates oxygen delivery to tissues.<sup>84</sup>

In one study, four patients who had a continuing need for transfusions while receiving hemodialysis treatment were found to have excessive splenic sequestration of erythrocytes. Marked improvement in red cell survival and disappearance of the transfusion requirement followed splenectomy.<sup>63</sup>

If a renal homograft is successful, both erythropoietic and excretory functions may

return.<sup>41,51,65,73,80</sup> Normal levels of erythropoietin often can be detected in plasma following a graft.<sup>41,51,80</sup> When the graft persists for two or more months, it is common to observe a reticulocyte response and an increase in VPRC (Fig. 19-5). Normal and even supra-normal<sup>65</sup> VPRC values ultimately may be achieved, but the hematologic response often is delayed until immunosuppressive measures can be reduced (Fig. 19-5). In one series, 80% of over 100 patients demonstrated

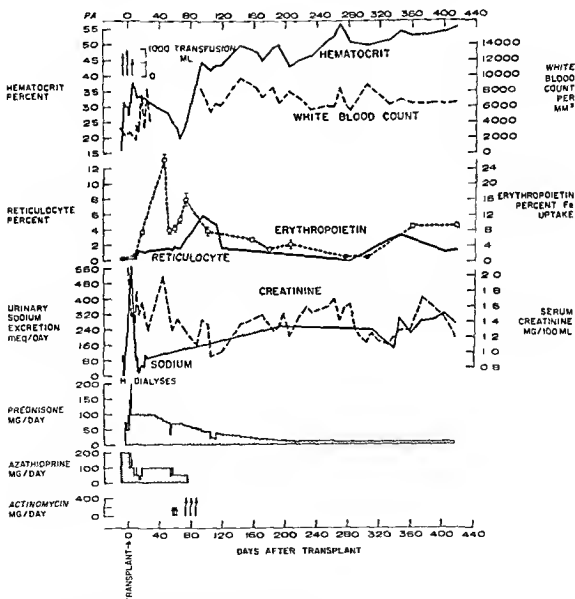


Fig 19-5. The response of the anemia of renal disease to renal transplantation (From Abbrecht and Greene,<sup>41</sup> courtesy of authors and *Annals of Internal Medicine*)

an increase in blood hemoglobin following renal homograft.<sup>65</sup> Failure to respond could usually be explained on the basis of hemorrhage, vigorous immunosuppression, or graft rejection. Interestingly, the rejection phenomenon often has been accompanied by increased erythropoietin levels.<sup>41 80</sup>

## Anemia in Cirrhosis and Other Liver Diseases

Anemia is a frequent manifestation of liver disease. It is particularly characteristic of Laennec's cirrhosis, and much of the information to follow has been derived from the study of patients with that disorder. In addition, however, changes in the structural characteristics of the red cells, reduction in red cell survival time, and even mild anemia have been observed in association with such entities as biliary cirrhosis,<sup>110</sup> hemochromatosis,<sup>116</sup> postnecrotic cirrhosis,<sup>116</sup> and acute hepatitis.<sup>121</sup> These studies will be cited where relevant.

Many etiologic factors have been implicated in the anemia associated with liver disease. Thus, *folate deficiency* may be present, especially in alcoholics (Chapter 14, page 578). Furthermore, even in the absence of liver disease, alcohol has been shown to exert a direct depressive effect on the bone marrow (Chapter 18, page 686). Iron deficiency also may develop in patients with liver disease as a result of blood loss, chiefly from the upper

gastrointestinal tract, but also from the nose, from hemorrhoids, and from menorrhagia because of a frequently associated disturbance of hemostasis (Chapter 38). In various series, blood loss occurred in from 24 to 70% of patients with alcoholic cirrhosis.<sup>116</sup> In addition, however, anemia may be found in association with liver disease in the absence of any of the above complicating factors. Although much remains to be learned regarding the pathogenesis of such "uncomplicated" anemia, it appears to result from a combination of hypervolemia, shortened red cell survival, and impaired ability of the marrow to respond to the anemia.

### Prevalence and Severity

Approximately 75% of patients with chronic liver disease are found to have anemia as defined by a reduction in the VPRC or hemoglobin level.<sup>101,116,122,123,125</sup> The whole blood volume, however, averages about 15% greater than normal (Table 19-3), and this hemodilution tends to exaggerate the anemia.<sup>116,118</sup> Thus, some patients may have a reduced VPRC even though the circulating red cell mass is normal. In one series, the incidence of anemia (VPRC less than 0.40 l/l) was 70%, but the circulating red cell mass was reduced in only 40% of the patients.<sup>122</sup> The magnitude of the hypervolemia in Laennec's cirrhosis appears to be related to the degree of portal hypertension, but not to the

Table 19-3. Changes in Blood Volume in Patients with Cirrhosis<sup>118</sup>

Measurement	Normal Subjects	Patients with Cirrhosis	
		Without Ascites	With Ascites
VPRC (l/l)	0.42	0.35	0.34
Red cell mass (ml/kg)	23	20	19
Plasma volume (ml/kg)	42	57	55
Whole blood volume (ml/kg)	65	74	74

Values are means of 24 normal subjects, 63 patients with cirrhosis and no ascites, and 34 patients with cirrhosis and ascites. In all groups there were about twice as many men as women.

presence or absence of ascites (Table 19-3).<sup>118</sup>

The degree of anemia is rarely severe in the absence of complications. In a series of 35 patients with alcoholic cirrhosis, the hemoglobin level averaged 12.3 g/dl, although it ranged from 6.8 to 15.8 g/dl.<sup>116</sup> Similar values have been reported by others.<sup>122,125</sup>

### Hematologic Findings

In its "uncomplicated" form, the anemia of liver disease probably is normocytic and normochromic. However, there are few observations on which such a judgment can be made, since, in most reported series, a population of patients with "uncomplicated" anemia has not been clearly identified. Occasionally the anemia is mildly macrocytic, but it is rare for the MCV to exceed 115 fl in the absence of megaloblastic changes in the bone marrow (Chapter 14). When there is complicating iron deficiency, hypochromia may be observed, but microcytosis is unusual.<sup>116</sup>

When true macrocytosis is found in patients with liver disease, it probably results either from complicating folate deficiency or from stimulated erythropoiesis.<sup>115</sup> The reported incidence of an increased MCV in patients with liver disease has varied from 33 to 65%.<sup>101,112,116,117</sup> Even more common is so-called "thin" macrocytosis, which may be defined as an increase in mean cell diameter with a normal mean cell volume (Chapter 14, page 567). In one study of 222 patients with various kinds of liver disease, an increased mean cell diameter was found in 137 (62%).<sup>102</sup> The cases were classified into three groups: "thin" macrocytosis (81 patients), "target" macrocytosis (39 patients), and "thick" macrocytosis (17 patients). The MCV was increased only in the last group.

The reticulocyte count often is increased. In a study of 16 patients, the maximum count averaged 8.6% and ranged from 2.3 to 24.6%.<sup>112</sup> The reticulocytosis can be suppressed by alcohol ingestion; therefore, if alcohol intake has continued until the patient is first seen, the reticulocyte count probably

will be low. Then, upon withdrawal of alcohol, an increase will be observed, with the maximum value occurring on about the seventh day.<sup>112</sup>

Mild thrombocytopenia is found in about half the patients with cirrhosis, but values less than  $50 \times 10^9/l$  are uncommon.<sup>101,122</sup> A variety of leukocyte abnormalities may be observed; in a study of 25 patients, 16 had lymphopenia, four had neutropenia, and 12 had neutrophilia.<sup>101</sup> Severe pancytopenia associated with splenomegaly in liver disease (Banti's syndrome) is discussed in Chapter 45.

Bone marrow cellularity is normal or increased.<sup>101,116,117</sup> Often erythroid hyperplasia, with a reduced M:E ratio, is observed. Red cell precursors at times have been described as "macronormoblasts," a term which implies that their size is increased but that their nuclear chromatin structure is normal.<sup>101,117,120</sup> In as many as 20% of the subjects, however, frank megaloblastosis may be present (Chapter 14, page 578).

### Erythrokinetics and Pathogenesis

Red cell survival tends to be moderately shortened in patients with alcoholic liver disease. When measured with the <sup>51</sup>Cr method (Chapter 5, page 197), erythrocyte survival was subnormal ( $t_{1/2}$  Cr less than 24 days) in 48 of 68 patients (70%) in four different studies.<sup>113,116,121,122</sup> The average  $t_{1/2}$  Cr in these patients was 20 days; in only 14 of them was the  $t_{1/2}$  Cr less than 16 days. Shorter survival was observed in a more anemic population of cirrhotic patients (mean hemoglobin 9.3 g/dl).<sup>112</sup> In them, the average  $t_{1/2}$  determined by the Ashby method (page 197) was 22.7 days and the range five to 49 days (normal, 52 to 68 days). The disappearance pattern was curvilinear, indicating random destruction. The rate of red cell disappearance correlated well with the degree of anemia. Erythrocyte survival is particularly likely to be reduced in patients with predominant indirect bilirubinemia, ie, when the indirect fraction is greater than 2.5 mg/dl and constitutes more than 70% of the

total.<sup>108</sup> Erythrocyte survival also has been found to be significantly shortened in patients with biliary cirrhosis,<sup>110</sup> obstructive jaundice, and infectious hepatitis,<sup>108,121</sup> even in the absence of anemia.

The reasons for the decreased red cell life span in liver disease have not been elucidated fully. Cross transfusion studies have demonstrated improved survival when patient cells were transfused to normal recipients, suggesting that the hemolytic factor is extracorporeal.<sup>111</sup> Jandl and associates suggested that sequestration and destruction of red cells in the often enlarged spleen plays a prominent role.<sup>112</sup> It is true that erythrocyte survival is found to be significantly shorter in patients with splenomegaly than in those without.<sup>116</sup> Furthermore, with the <sup>51</sup>Cr technique, excessive splenic sequestration has been demonstrated in some patients<sup>108,110,112,127</sup>; but in the majority, splenic uptake was normal even though erythrocyte survival was reduced.<sup>116</sup> In a few patients, but not all, splenectomy has been followed by correction of the hemolytic process.<sup>101,111,122</sup>

Characteristic alterations in red cell membrane lipids are found in patients with hepatitis, cirrhosis, and obstructive jaundice<sup>109,106,119,123</sup> (Fig. 19-6). In the usual

case, there is a 25 to 50% increase in both cholesterol and lecithin in the membrane. These changes result in an increased cell surface area, leading to the characteristic "thin macrocyte" or target cell. There is no good evidence, however, that such abnormalities result in reduced cell survival. In contrast, in a few patients with severe hepatocellular disease, an accumulation of membrane cholesterol occurs without a corresponding increase in lecithin. This is associated with the formation of so-called "spur cells," erythrocytes which are covered with spike-like projections and which probably are morphologically indistinguishable from the acanthocytes seen in abetalipoproteinemia (page 762).<sup>106,121,126</sup> The appearance of spur cells is accompanied by pronounced shortening of erythrocyte survival, possibly because the distorted cells become trapped in the reticuloendothelial system. The alteration in red cell lipids in liver disease is not fully understood, but at least three factors have been implicated: (1) reduced LCAT activity (Chapter 3), (2) retention of bile salts, and (3) increase in the plasma free cholesterol to phospholipid ratio.<sup>106</sup> Spur-cell hemolytic anemia can be induced in vivo by administration of lithocholic acid,<sup>107</sup> but a pathogenetic relation between this bile-acid deriv-

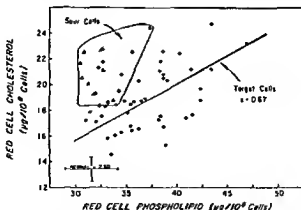


Fig. 19-6 Erythrocyte membrane lipids in liver disease. Normal values are shown in the lower left-hand corner. In patients with target cells the degree of increase in cholesterol correlates well with that in phospholipids. In "spur cell" anemia, only cholesterol is increased. (From Cooper and Jandl,<sup>104</sup> courtesy of the authors and Journal of Clinical Investigation.)



ative and naturally occurring spur-cell anemia has not been established.

Zieve distinguished a special group of patients with alcoholic liver disease and shortened red cell survival.<sup>129</sup> They had mild impairment of hepatic function, and fatty infiltration was the predominant histologic finding. Plasma triglycerides were markedly increased. Both the shortened  $t_{1/2}$  and the liver disease tended to be transient. Recovery was spontaneous, but recurrence on exposure to alcohol was not unusual. Whether this syndrome represents a distinct pathophysiologic entity or a collection of findings occurring together by chance remains uncertain. In particular, there is no direct evidence that the triglyceridemia induces hemolysis.<sup>123</sup> Furthermore, red cell lipids in patients with "Zieve's syndrome" are no different than those found in other cirrhotic patients.<sup>105</sup>

In addition to the shortened erythrocyte survival, some studies have suggested that in patients with liver disease the marrow response to the anemia is inadequate. For example, plasma iron turnover, red cell iron utilization, and erythrocyte iron turnover were normal or reduced in the majority of patients in one series.<sup>116</sup> In another study, however, these parameters were increased two- to threefold<sup>103</sup> (Table 19-2). The presence or absence of complications may explain such discrepancies. Alcohol, in particular, will depress erythropoiesis, and if the patient is studied before the effects of alcohol ingestion have subsided, marrow function will appear to be depressed.

Another possible explanation has been advanced for impaired erythropoiesis in liver disease. It is possible that one of the functions of the normal liver is the synthesis of an erythropoietin precursor that can be activated by interaction with a renal enzyme (Chapter 4, page 181). The erythropoietin response to hypoxia was found to be markedly impaired in hepatectomized or partially hepatectomized rats as compared to sham-operated controls.<sup>114</sup> Additional evidence for a hepatic erythropoietic factor is found in certain patients who develop erythrocytosis with hepatic carcinoma.<sup>109</sup> It is possible, therefore,

that a diseased liver makes inadequate amounts of this precursor. As yet, there is no direct proof for this hypothesis.

## Anemias Associated with Endocrine Disorders

Anemia commonly accompanies disorders affecting the thyroid gland, adrenal glands, gonads, or pituitary gland. In general the anemia is mild to moderate in degree and produces no symptoms. In fact, the amount of circulating hemoglobin may be entirely appropriate to the needs of the organism because the metabolic disturbance often results in reduced oxygen requirements. In most patients, these endocrine disorders begin insidiously and the early symptoms are nonspecific and include fatigue. Consequently, when the initial investigation detects a reduced hemoglobin level, the symptoms may be ascribed to the anemia and the diagnostic studies may then be directed toward the hematopoietic system. Few morphologic abnormalities will be observed and none is specific. Unless the clinician suspects endocrine disease, the diagnosis may be overlooked.

### Hypothyroidism

In various reported series, anemia has been observed in 21 to 60% of patients with hypothyroidism.<sup>118</sup> Three morphologic types of anemia have been described in these patients.<sup>145,148,174,195</sup> The relative incidence of the three types differed considerably in two large series reported since 1960 (Table 19-4). The reasons for the differences are not clear, but they may have been related to factors not directly concerned with the thyroid disease as well as to its degree and duration (see below). When *hypochromic, microcytic anemia* was found in association with myxedema it was due to iron deficiency.<sup>145,182,198</sup> It responded to iron therapy even if thyroid hormone was not administered, and was not relieved by hormone therapy if iron was withheld. Iron deficiency occurs in association with myxedema partly because menor-



Fig 19-7. Photographs of a girl of 15 years with "refractory" anemia, before and four months after therapy with desiccated thyroid. Note the pale, puffy, pasty appearance before therapy.

rhagia is a frequent manifestation of the illness,<sup>169</sup> and partly also, perhaps, because the achlorhydria found in myxedema subjects<sup>198</sup> may lead to impaired absorption of food iron (Chapter 4, page 156). Distinctly *macrocytic anemia* usually results from complicating deficiency of vitamin B<sub>12</sub><sup>193</sup> or folate.<sup>174</sup> The increased incidence of pernicious anemia in thyroid disease possibly is the result of an autoimmune mechanism, as discussed in Chapter 15 (page 606). Folate deficiency may

have developed because of an inadequate diet, but this could not be established with certainty.<sup>174</sup>

If the patients with iron, folate, or vitamin B<sub>12</sub> deficiency are excluded, there remains a significant population of hypothyroid patients with anemia. This type of anemia constitutes the so-called "uncomplicated anemia of hypothyroidism,"<sup>143,174,198</sup> and is a manifestation of the hormone deficiency itself. The anemia usually is mild, the VPRC rarely falling below 0.35 l/l. However, the plasma volume often is decreased,<sup>167,188,199</sup> a change which tends to minimize the reduction in VPRC. The degree of anemia is related to both the severity and duration of the hypothyroidism.<sup>196</sup> It was observed that the VPRC continued to fall for as long as six months after thyroidectomy in previously euthyroid subjects, even though the basal metabolic rate remained at a stable, reduced level. In man, the "uncomplicated" anemia usually is normocytic and normochromic, but may be slightly macrocytic.<sup>148,174,198</sup> It is macrocytic in rabbits<sup>180</sup> and rats<sup>170,193</sup> and normocytic in dogs.<sup>175</sup> Anisocytosis, poikilocytosis, or other red cell morphologic abnor-

Table 19-4. The Incidence of Various Types of Anemia in Patients with Myxedema from Two Different Studies

Type of Anemia	Sheffield, England <sup>198</sup>	University of Virginia <sup>148</sup>
Normocytic normochromic	4%	26%
Hypochromic, microcytic	14%	3%
Macrocytic	13%	14%
All anemias	31%	43%

malities are not impressive, and usually the leukocyte and platelet counts are within the normal range, although both may be slightly reduced.<sup>196</sup> The bone marrow may be mildly hypoplastic, but there is no significant alteration in the M:E ratio.<sup>142,178</sup>

From a kinetic viewpoint, the anemia of myxedema seems to be entirely explained by reduced red cell production. With one exception,<sup>147</sup> reported studies have indicated that erythrocyte survival is normal or even slightly prolonged in man<sup>179,184,191,198</sup> and in dogs,<sup>149</sup> but plasma iron transport and erythrocyte iron turnover rates are reduced, indicating *subnormal red cell production*<sup>58,175,179</sup> (Table 19-2).

Bornford was the first to suggest that the anemia of myxedema was "adaptive" in nature, i.e., a physiologic adjustment to the reduced needs of the organism for oxygen.<sup>145</sup> Since that time, this hypothesis has become even more tenable because of what has been learned about the erythropoietin-secreting mechanism and its relation to tissue oxygen tension (Chapter 4). There also is experimental evidence consistent with it. For example, the marrow of the isolated, hind limb of the dog exhibits an erythropoietic response to perfusion with erythropoietin, but not to L-triiodothyronine (T<sub>3</sub>),<sup>162</sup> an observation which suggests that the role of the latter hormone is indirect. Furthermore, administration of 2,4 dinitrophenol, a drug which increases oxygen consumption without improving thyroid function, causes an increase in red cell mass in hypothyroid patients<sup>188</sup> and in thyroidectomized rats.<sup>160</sup> Finally, hyperoxia can inhibit the erythropoietic response to T<sub>3</sub>.<sup>164</sup> However, the observation that non-calorigenic D-isomer of triiodothyronine can stimulate erythropoiesis without alternating oxygen consumption has been cited as evidence for a hormonal effect that is not oxygen dependent.<sup>157,175,186</sup>

The response of the anemia of hypothyroidism to thyroid hormone is sluggish. Characteristically, there is no distinct reticulocytosis, and the VPRC returns to normal only gradually over about a six-month period (range three to 12 months).<sup>145,148,198</sup>

## Hyperthyroidism

The circulating red cell mass usually is increased in patients with hyperthyroidism<sup>167,185</sup>; however, the VPRC most often remains within normal limits because the plasma volume also increases. In general, the rate of erythropoiesis is increased both in hyperthyroid patients<sup>156</sup> and in experimental animals.<sup>200</sup> Erythrocyte 2,3 diphosphoglycerate (2,3 DPG) is increased, resulting in reduced oxygen affinity,<sup>187,194</sup> changes which are appropriate to increased tissue oxygen utilization (Chapter 3).

Anemia is uncommon in hyperthyroidism; however, in one study, among 50 patients with hyperthyroidism, four women were found with moderately severe anemia (VPRC, 0.27 to 0.30 l/l) for which no cause could be found other than the metabolic disorder.<sup>190</sup> Furthermore, the anemia was corrected when the hyperthyroidism was treated. In the anemic patients, the hyperthyroidism was of unusual severity and prolonged duration. Mild anisocytosis and poikilocytosis were present and slight hypochromia was apparent in three of the four. Impaired iron utilization was found on ferrokinetic study, suggesting that erythropoiesis was ineffective.

## Addison's Disease

In 28 patients with untreated Addison's disease, the average blood hemoglobin level was 13.2 g/dl and the range was 9.4 to 18 g/dl.<sup>143</sup> The red cells were normocytic and normochromic. Shortly after hormonal replacement therapy was begun, various measures of red cell concentration fell about 20%—the average hemoglobin from 13.7 to 10.7 g/dl and the VPRC from 0.416 to 0.325 l/l. It was concluded that the red cell mass is reduced in Addison's disease, but that the change is partially obscured by the dehydration that usually accompanies the condition. Treatment corrected the dehydration and restored the plasma volume, thereby making apparent the true degree of anemia. Later in the course,

reticulocytosis and return to normal hemoglobin levels were observed.

Following adrenalectomy in rats maintained on saline solution, a mild anemia develops.<sup>151,171,199</sup> The blood hemoglobin drops about 1.5 g/dl in three weeks. The anemia is corrected by administration of adrenal corticoids.

### Hypogonadism

After the age of puberty, values for the VPRC, blood hemoglobin concentration, and red cell count average about 10 to 13% higher in men than in women (see appendix A). In eunuchoid or castrated men, the values for these measures fall to within the normal female range.<sup>141,185</sup> Urinary erythropoietin levels in normal men are about three times those found in women.<sup>110</sup>

The differences between the sexes are accounted for chiefly by the stimulating effect of androgens on erythropoiesis. In addition, some observations suggest that estrogens exert a suppressive effect. Thus, castration of male rats is followed by a decrease in hemoglobin, whereas castration of female rats brings about an increase.<sup>152,168,195</sup> The administration of androgens to castrated males corrects the anemia. Androgens can also stimulate erythropoiesis in normal subjects. In normal men, testosterone enanthate induced an average increase in the total red cell mass from 1.7 to 2.3 liters.<sup>165</sup> The increase in VPRC was of smaller magnitude, from 0.456 to 0.494 l/l, probably because the plasma volume also increased. Androgens act by increasing renal synthesis of erythropoietin (or the renal erythropoietic factor).<sup>141,172</sup> Estrogens produce anemia when given in large amounts to rats,<sup>158,189</sup> possibly by suppressing hepatic synthesis of the serum substrate for erythropoietin<sup>172</sup> (Chapter 4).

### Hypopituitarism

Moderately severe, nonprogressive anemia is a well-recognized feature of pituitary insufficiency, regardless of cause. In an extensive review of 595 reported cases of Sim-

mond's disease, published in 1942, the average blood hemoglobin concentration was found to be 67 "percent of normal" (approximately 10 g/dl),<sup>159</sup> and the range was 21 to 103 "percent" (3 to 16 g/dl). Similar values were found in patients whose hypopituitarism arose from neoplasms.<sup>154,173</sup> Anemia may also be found in prepubertal pituitary dwarfs<sup>177</sup>; however, the degree of reduction of circulation red cell volume may be obscured by contraction of the plasma volume.<sup>192</sup>

The anemia usually is normocytic and normochromic, and the red cells appear morphologically normal. In some patients, slight hypochromia or macrocytosis has been observed<sup>151,197</sup>; however, complicating deficiencies of iron or folate were not excluded. Kinetic studies have demonstrated reduced red cell production.<sup>155,177</sup>

The anemia of hypopituitarism probably results from deficiencies of the hormones of target glands controlled by the pituitary, especially the thyroid and adrenal hormones, but also androgens. In addition, lack of other pituitary factors, such as growth hormone,<sup>153,163,168,177,192</sup> prolactin,<sup>178</sup> or other poorly characterized pituitary principles<sup>183</sup> may be of importance. The interrelations of these various hormones have been studied in experimental animals, especially the rat. In this species, hypophysectomy results in a moderately severe, slightly hypochromic and microcytic anemia<sup>144,153</sup> that is associated with a pronounced decrease in erythroid elements in the bone marrow.<sup>146</sup> There is no hemolysis; in fact, red cell survival is prolonged.<sup>181</sup> Treatment with a combination of thyroxine, cortisone, and growth hormone corrects both the anemia and the marrow hypoplasia<sup>153</sup> and such treatment is more effective than any single hormone given by itself. Combined adrenalectomy and thyroidectomy results in an anemia that is similar, but not identical, to that found after hypophysectomy.<sup>150,153</sup>

As is the case with the anemia of hypothyroidism (page 711), panhypopituitarism probably produces its effects on erythropoiesis chiefly by reducing tissue oxygen consumption.<sup>153</sup> The organism reacts to this

decreased need for oxygen by secreting less erythropoietin, and the red cell mass diminishes until a new equilibrium between oxygen supply and demand is established. This hypothesis is supported by the observations that (1) tissue oxygen consumption is low in the hypophysectomized rat, even if the red cell mass is restored to normal<sup>153</sup>; (2) once equilibrium has been established, the marrow of the hypophysectomized animal will respond to hypoxia, bleeding, or cobalt administration<sup>153,161</sup>; (3) correlation between oxygen consumption and rate of erythropoiesis can be demonstrated in hypophysectomized animals given thyroxine or 2,4 dinitrophenol<sup>153</sup>; and (4) erythrocyte 2,3 DPG levels, which increase when tissue oxygen delivery is compromised, are normal in patients with panhypopituitarism.<sup>182</sup>

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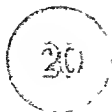
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## *The Hemolytic Disorders: General Considerations*

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develops because there is an additional pathogenetic factor, namely, failure of the marrow to increase red cell production sufficiently to compensate for the shortened survival of the red cells ("relative marrow failure") (Chapter 19). These illnesses have a *hemolytic component*, but it is misleading to include them with the hemolytic anemias.

More narrowly defined, the term *hemolytic anemia*, or better still *hemolytic disorders*, is limited to those conditions in which the rate of red cell destruction is accelerated and the ability of the bone marrow to respond to the stimulus of anemia is unimpaired. Such a definition is of practical as well as semantic importance. One may expect hemolytic disorders as here defined to be associated with features that serve to set them apart from other forms of anemia, namely, signs of accelerated erythrocyte destruction together with vigorous blood regeneration. In general, a similar picture is not observed in anemias with a mild hemolytic component and relative marrow failure. Once a clearly hemolytic process has been detected, the diagnostic possibilities are reduced, and an orderly plan of investigation leading to a specific diagnosis can be formulated.

The distinction between frankly hemolytic anemias and those associated with relative marrow failure can best be understood in quantitative terms (Table 20-1). Under

**Definitions**

Hemolytic anemias are anemias that result from an increased rate of red cell destruction. Strictly interpreted, however, such a definition would include a number of common but kinetically complex anemias, such as the anemia of chronic disorders and of renal disease, anemia associated with vitamin B<sub>12</sub> or folate deficiency, and even iron-deficiency anemia. In these, the rate of red cell destruction is modestly increased, but not to a degree that would, by itself, lead to anemia. The anemia

Table 20-1. Representative Values for Rates of Hemoglobin Production and Destruction in Several Types of Anemia

	Hemoglobin Concentration (g/dl)	Total Circulating Hemoglobin* (g)	Erythrocyte Survival (days)	Hemoglobin Produced and Destroyed† (g/day)
Normal subject	16.7	800	120	6.7
Relative marrow failure	10	480	60	8.0
Compensated hemolytic state	16.7	800	20	40
Hemolytic anemia	10	480	12	40

\*Based on an assumed blood volume of 4.8 l

†Calculated from the ratio  $\frac{\text{Total Circulating Hemoglobin (g)}}{\text{RBC Survival (days)}}$ 

maximal stimulation, the normal marrow is capable of undergoing hyperplasia until its production rate is increased about six- to eight-fold.<sup>7</sup> With optimal marrow compensation, it is theoretically possible for the survival of red cells in the circulation to decrease from the normal 120 days to as little as 15 to 20 days without anemia developing. Such an increase in both destruction and production of erythrocytes can produce a compensated hemolytic state without anemia being present, usually known as *compensated hemolytic disease*. On the other hand, when red cell survival becomes so short that anemia develops in spite of a vigorous erythropoietic response, the term *hemolytic anemia* is appropriate.

## Development of Knowledge Concerning the Hemolytic Anemias

Galen, in AD 150, described a patient who developed jaundice after being bitten by a viper.<sup>10</sup> He postulated that, in this and in certain other cases, jaundice was not the result of liver disease, but instead was of splenic origin. In the modern era, Vanlair and Masius were probably the first to report a patient with hemolytic anemia,<sup>23</sup> although the dark urine of paroxysmal nocturnal hemoglobin-

uria had been noted even earlier (page 934). Their patient had anemia, acholuric jaundice, splenomegaly, and abnormally small red cells (presumably spherocytes), and they termed the condition "microcythemia." They proposed that the jaundice arose from exaggerated destruction of erythrocytes and transformation of the liberated hematin to bilirubin.

In 1890 and 1893, Wilson and Stanley reported, in six members of a single kindred, the occurrence of a chronic condition characterized by splenomegaly, icterus, a predisposition to gallstones, and, in one of the members, progressive anemia resulting in death.<sup>23</sup> Almost certainly, the disease they described is the one we know today as hereditary spherocytosis (Chapter 21). Around the turn of the century, Minkowski<sup>19</sup> and Chauffard<sup>1</sup> published comprehensive descriptions of this congenital form of hemolytic anemia and Chauffard called attention to the increased osmotic fragility of the erythrocytes. The term "hemolytic" was probably coined by William Hunter, who used it to distinguish anemias caused by excessive blood destruction from "hemogenic" anemias, such as pernicious anemia.<sup>14</sup>

Hayem, in 1898,<sup>13</sup> and, later, Widal, Abrami, and Brulé<sup>24</sup> (1907-1909) pointed out that, whereas the classic congenital hemolytic anemia described by Minkowski

and Chauffard often caused few symptoms, another type, which they regarded as acquired, frequently was associated with severe anemia and profound illness. In the acquired type they included cases of excessive blood destruction associated with various infections or intoxications, as well as cases of unknown cause. In the latter, autohemagglutination was often noted. Chauffard and Troisier<sup>5</sup> were able to demonstrate autohemolysins in the serum of a few patients with acute, acquired hemolytic anemia, in contrast to their absence in the congenital cases, and spoke of "hemolytic icterus."

Despite these studies, doubt persisted for many years that there is a truly "acquired" form of hemolytic jaundice. Apparently acquired cases commonly were ascribed to aggravation of a latent, congenital illness. Some 20 years passed before Lederer<sup>17</sup> and Brill<sup>2</sup> called attention to an acute, idiopathic hemolytic anemia which was associated with infectious disease and from which rapid recovery ensued following blood transfusions. Subsequently, similar cases attracted attention,<sup>11</sup> and the fact that they differed from cases of congenital hemolytic jaundice in crisis ultimately came to be recognized. The existence of acquired hemolytic anemia in contrast to the congenital form was clearly established by Dameshek and Schwartz,<sup>8</sup> who demonstrated abnormal hemolysins in the blood of patients suffering from acute hemolytic anemia and showed that spherocytosis and increased osmotic fragility can develop during the course of such anemia in man as well as in acute hemolytic anemia produced experimentally in animals.

The subsequent development of the field can be credited in large part to the growth of understanding of the metabolism of the red corpuscle (Chapter 3), the structure and function of its membrane, and the abnormalities resulting from alterations in the hemoglobin molecule (Chapter 24). Important advances were also made by the application of serologic techniques to the study of hematologic disorders.

Between 1930 and 1950, several cases of congenital hemolytic anemia which differed

from classic hereditary spherocytosis in that there was no spherocytosis, erythrocyte osmotic fragility was normal, and splenectomy was not therapeutically beneficial, were reported.<sup>1,6,12,16,21</sup> Crosby<sup>6</sup> and Kaplan and Zuelzer<sup>16</sup> applied the term "congenital (or familial) non-spherocytic hemolytic anemia" to such cases. It soon became apparent that this designation did not refer to a single disease entity. The autohemolysis test of Selwyn and Dacie<sup>20</sup> (page 735) served not only to detect some of these illnesses, but also to separate them into two categories, type I and type II. Results of the test also suggested that in type II disease glycolysis might be defective. The discovery by Carson et al<sup>3</sup> of glucose-6-phosphate dehydrogenase deficiency, followed by the demonstration by Valentine and coworkers<sup>22</sup> that a specific glycolytic enzyme (pyruvate kinase) was deficient in the red cells of patients with type II congenital nonspherocytic hemolytic anemia, led to an intensive search for, and discovery of, many other red cell enzymatic defects associated with hemolytic disease (Chapters 22, 23).

## Pathogenesis and Classification

The disorders associated with hemolytic anemia have been classified in various ways, none of which is entirely satisfactory. On clinical grounds they have been divided into *acute and chronic forms*, but such a division is of limited usefulness since acute episodes may develop during the course of chronic disorders. Of somewhat greater utility is a classification based on the site of hemolysis, ie, whether it is predominantly within the circulation ("intravascular") or within tissue macrophages ("extravascular"). Since the intravascular hemolytic disorders are accompanied by unique manifestations, such as hemoglobinemia, hemoglobinuria, and hemosiderinuria, the classification is easily made and often serves to limit the diagnostic possibilities<sup>83</sup> (Table 20-2). On the other hand, since most hemolytic diseases are characterized by extravascular red cell destruction,

**Table 20-2. Hemolytic Anemias Characterized by Predominantly Intravascular Red Cell Destruction**

- 1 Paroxysmal nocturnal hemoglobinuria (Chapter 29)
- 2 Disorders associated with erythrocyte fragmentation (Chapter 28)
- 3 Certain immunohemolytic anemias (Chapter 27)
  - a Transfusion reactions resulting from ABO iso-antibodies
  - b Paroxysmal cold hemoglobinuria
  - c Some instances of idiopathic autoimmune hemolytic anemia
- 4 Those associated with certain infections
  - a Blackwater fever in *falciparum* malaria
  - b Clostridial sepsis
- 5 Those caused by certain chemical agents
  - a Intravenous administration of distilled water
  - b Snake and spider venoms
  - c Arsenic poisoning
  - d Acute drug reactions in association with glucose-6-phosphate dehydrogenase deficiency
- 6 Thermal injury

detection of this mode of hemolysis still leaves one with a large number of diagnostic possibilities.

Hemolytic disorders also may be divided into inherited and acquired varieties. This division forms the basis for a classification that, as a rule, is the most useful to the clinician (Table 20-3). The classification also has pathogenetic significance, for the fundamental nature of hereditary lesions is quite different from that of acquired ones. Excessive destruction of erythrocytes may occur either because of an *intrinsic* defect in the cell itself or because of the action of *extrinsic* agents on normally constructed cells. As a general rule, the intrinsic defects are *inherited* and the extrinsic ones are *acquired*. There are only a few known exceptions to this generalization; these include (1) paroxysmal nocturnal hemoglobinuria (Chapter 29), an acquired disorder characterized by an *intrinsic* red cell defect; (2) certain inherited intrinsic defects (eg, the most common form of glucose-6-phosphate dehydrogenase deficiency) that are associated with no ill effects in the absence of an extrinsic agent, usually a drug (Chapter 23); and (3) thermal injury, which

induces an acquired, *intrinsic* erythrocyte defect.

Intrinsic and extrinsic abnormalities have been distinguished from one another by performing cross-transfusion erythrocyte survival studies (Table 20-4). Thus, it was shown that when normal erythrocytes were transfused to patients in whom there was an extrinsic cause for hemolysis the donated cells were destroyed as rapidly as the patient's own. If, on the other hand, the patient's corpuscles were removed from their unfavorable environment and were transfused to a normal recipient their survival time was normal.<sup>17</sup> In contrast, when the disorder was due to an intrinsic defect of the red cells the patient's cells, when given to a normal recipient, were disposed of more rapidly than those of the recipient; the latter's erythrocytes, if transfused into the patient, maintained a normal "life span." Such cross-transfusion experiments are not required in the study of the great majority of hemolytic anemias today, but they served to clarify the pathogenesis of hemolytic anemias before more readily applicable investigation procedures had been devised.

The inherited, intrinsic disorders of the erythrocyte can be subdivided into five groups, depending upon which major erythrocyte structure or metabolic pathway is impaired. Thus, there are defects affecting the membrane, the glycolytic pathway, glutathione metabolism, and the hemoglobin molecule (Table 20-3). There is also a small, miscellaneous group of disorders resulting from deficiencies of enzymes not associated with glycolysis or glutathione metabolism.

The acquired hemolytic anemias may be subdivided into eight groups, depending upon the nature of the extrinsic hemolytic factor. These factors include antibodies, physical trauma, infectious agents, physical agents, chemical agents, hypophosphatemia,<sup>15</sup> and liver disease ("spur cell anemia," Chapter 19). A separate category must be provided for paroxysmal nocturnal hemoglobinuria, which is of unknown cause, but, as noted previously, is unique in that it is due to an intrinsic abnormality of the red cell.

Table 20-3. Etiologic and Pathogenetic Classification of the Hemolytic Disorders

- I Inherited hemolytic disorders**
- A Defects in the erythrocyte membrane (Chapter 21)**
- 1 Hereditary spherocytosis ✓
  - 2 Hereditary elliptocytosis ✓
  - 3 Abetalipoproteinemia (acanthocytosis)
  - 4 Hereditary stomatocytosis ✓
  - 5 Lecithin-cholesterol acyl transferase (LCAT) deficiency
  - 6 Defective phospholipid fatty acid transfer
- B Deficiency of erythrocyte glycolytic enzymes (Chapter 22)**
- 1 Pyruvate kinase ✓
  - 2 Hexokinase
  - 3 Glucose phosphate isomerase
  - 4 Phosphofructokinase
  - 5 Aldolase
  - 6 Triosephosphate isomerase
  - 7 2,3-Diphosphoglyceromutase
  - 8 Phosphoglycerate kinase
  - 9 Enolase
- C Deficiencies of enzymes involved in the pentose phosphate pathway and in glutathione metabolism (Chapter 23)**
- 1 Glucose-6-phosphate dehydrogenase (G6PD)
  - 2 Glutathione reductase ✓
  - 3 Glutathione peroxidase ✓
  - 4 Glutathione synthetase ✓
  - 5 Glutamyl-cysteine synthetase
- D Deficiency of miscellaneous erythrocyte enzymes (Chapter 22)**
- 1 Adenylate kinase ✓
  - 2 Ribosephosphate pyrophosphokinase ✓
  - 3 Adenosine triphosphatase (ATPase)
- E Defects in globin structure and synthesis**
- 1 Unstable hemoglobin disease (Chapter 24)
  - 2 Sickle cell anemia (Chapter 25)
  - 3 Other homozygous hemoglobinopathies (CC, DD, EE—Chapter 25)
  - 4 Thalassemia major (Chapter 26)
  - 5 Hemoglobin H disease (Chapter 26)
  - 6 Doubly heterozygous disorders (hemoglobin SC disease, sickle-thalassemia, etc) (Chapters 25, 26)
- II Acquired hemolytic anemias**
- A Immuno-hemolytic anemias (Chapter 27)**
- 1 Transfusion of incompatible blood ✓
  - 2 Hemolytic disease of the newborn ✓
  - 3 Autoimmune hemolytic anemia due to warm-reactive antibodies ✓
    - a Idiopathic ✓
    - b Secondary ✓
      - (1) Virus and mycoplasma infections
      - (2) Lymphosarcoma, chronic lymphocytic leukemia
      - (3) Other malignant diseases
      - (4) Immune-deficiency states
      - (5) Systemic lupus erythematosus and other autoimmune disorders
- c Drug induced**
- 4 Autoimmune hemolytic anemia due to cold-reactive antibodies**
- a Cold hemagglutinin disease
    - (1) Idiopathic
    - (2) Secondary
  - b Paroxysmal cold hemoglobinuria
- B Traumatic and microangiopathic hemolytic anemias (Chapter 28)**
- 1 Prosthetic valves and other cardiac abnormalities
  - 2 Hemolytic-uremic syndrome
  - 3 Thrombotic thrombocytopenic purpura
  - 4 Disseminated intravascular coagulation
  - 5 Associated with immunologic phenomena (graft rejection, immune complex disease, etc)
- C Infectious agents (page 740)**
- 1 Protozoans malaria toxoplasmosis leishmaniasis
  - 2 Bacteria bacterial sepsis clostridial infection, cholera typhoid fever and others
- D Chemicals drugs and venoms**
- 1 Oxidant drugs and chemicals (see also Table 23 2, page 785)**
- a Naphthalene
  - b Nitrofurantoin
  - c Sulfonamides
  - d Sulfones
  - e Para-aminosalicylate
  - f Phenacetin
  - g Phenylsemicarbazide
  - h Resorcin
  - i Phenylhydrazine
  - j Aniline
  - k Hydroxylamine
  - l Nitrobenzene
  - m Phenol derivatives
  - n Chlorates
  - o Molecular oxygen
- 2 Nonoxidant chemicals**
- a Arsenic
  - b Copper
  - c Water
- E Physical agents**
- 1 Thermal injury
  - 2 (?) Ionizing irradiation
- F Hypophosphatemia**
- G "Spur-cell" anemia in liver disease**
- H Paroxysmal nocturnal hemoglobinuria**

**Table 20-4. The Cross-Transfusion Erythrocyte Survival Technique for Distinguishing Intrinsic and Extrinsic Erythrocyte Abnormalities**

Donor	Recipient	Erythrocyte Survival	
		<i>Intrinsic abnormality</i>	<i>Extrinsic abnormality</i>
Patient	Normal subject	Reduced	Normal
Normal subject	Patient	Normal	Reduced

Most of these different forms of hemolytic anemia will be discussed in the chapters that follow. In this chapter, those clinical and laboratory aspects that apply to all forms of hemolytic anemia will be considered along with an approach to the utilization of such information for detection of hemolytic anemia and for arriving at a specific etiologic diagnosis. Finally, the acquired hemolytic anemias resulting from infectious, chemical, and physical agents and hypophosphatemia will be discussed.

## Clinical Manifestations

Although there are a large number of hemolytic disorders (Table 20-3), the clinical features of hemolysis associated with them are much the same. In general, the manifestations of hemolytic anemia depend upon the duration of the process as well as its severity. Thus, chronic congenital hemolytic anemia can usually be distinguished from acute acquired hemolytic anemia on clinical grounds, as will be indicated below. When the onset of acquired hemolytic anemia is insidious, however, the distinction may be more difficult.

### Chronic Congenital Hemolytic Anemia

The major clinical features of congenital hemolytic anemia are related to anemia, jaundice, the occurrence of crises, splenomegaly, and the development of cholelithiasis. Less common manifestations include chronic leg ulcers and bony abnormalities.

### Manifestations of Anemia

The severity of the anemia varies greatly from one patient to another, even among those who have the same illness. Those with severe disease ordinarily are detected shortly after birth or within the first year of life. Severe pallor and the cardiovascular manifestations of anemia (Chapter 13) are the usual findings, and the latter may be serious enough to require treatment with blood transfusions.

More commonly, anemia is moderate to mild because the shortened erythrocyte survival is partially compensated for by increased activity of the bone marrow. Often the patients accommodate themselves remarkably well to the anemia, and there may be few symptoms. A moderately pallid complexion may be the only sign. Under such circumstances, detection frequently is delayed until later in childhood.

Finally, some patients have no anemia at all. The disease may then remain unsuspected until late in adult life, unless jaundice, a "crisis," or complications of gallbladder disease draw attention to the conditions. Sometimes such cases are only discovered in the course of a family study.

### Jaundice

In some instances, jaundice is first noted in the neonatal period.<sup>47,49</sup> Indeed, the anemia and jaundice may become so intense that immunohemolytic disease of the newborn (page 895) is simulated, kernicterus is feared, and an exchange transfusion is performed.<sup>47</sup> In many older children and adults with con-

genital hemolytic anemia, icterus is absent or mild enough to pass unnoticed. Careful inquiry may elicit a history of episodes of jaundice associated with trivial infections or unusual exertion. In others, jaundice is persistent, but never becomes intense (see page 726). Often slight scleral icterus is the only sign of hemolytic disease, and the description "more yellow than sick" has been appropriately applied to such patients.

The jaundice of hemolytic disease is *acholuric*; the bilirubin, being unconjugated, is not excreted into the urine (page 211). Furthermore, pruritus is absent. These features help to distinguish the icterus of hemolytic disease from that found with disorders of the hepatobiliary system.

### Crises

In the chronic congenital hemolytic diseases, long periods of relatively asymptomatic disease may be punctuated by episodes of acute anemia, icterus, or other manifestations of a so-called "crisis." Usually these episodes appear to be precipitated by an intercurrent infection, especially an upper respiratory infection<sup>34</sup>; "epidemics" have been reported in which several family members were affected simultaneously.<sup>37,45,46</sup> Most often, such crises result from transient failure of red cell production<sup>34,37,39,45</sup> ("aplastic" crisis), and the abrupt development of reticulocytopenia is a characteristic feature (Fig. 20-1). Mild leukopenia and thrombocytopenia may accompany the episode.<sup>37,45</sup> The bilirubin level does not increase and may even decrease somewhat. The aregenerative phase usually lasts 5 to 12 days.

Less commonly, crises may result from an increase in the rate of red cell destruction, possibly because of increased splenic activity ("hemolytic crisis").<sup>36,41</sup> In such episodes the degree of jaundice and the reticulocyte count increase and the spleen may enlarge. A third type of crisis comes about because of complicating folate deficiency ("megaloblastic crisis"), to which patients with chronic hemolysis appear to be particularly prone (Chapter 14, page 579). The onset of megaloblastic

crises tends to be more gradual than that of the other two and is unrelated to complicating infections.

If the clinician sees the patient for the first time because of a crisis, the sequence of events may well suggest an acute disease of recent onset rather than a chronic, congenital illness.

### Splenomegaly

The spleen commonly is enlarged in patients with congenital hemolytic anemias, except for those with sickle cell anemia (page 831). Most often the degree of enlargement is mild to moderate, but occasionally the spleen is huge. At times the splenomegaly leads to discovery of the disease because the spleen is detected on a routine physical examination. Alternatively, the spleen may attract attention because of a vague sensation of oppression or weight in the left side of the abdomen, or, less commonly, because it is the site of an attack of pain.

### Cholelithiasis

The development of gallstones and complications therefrom may play a prominent role in the clinical manifestations of congenital hemolytic anemias.<sup>31,42,50</sup> Symptoms of gallbladder disease may be the initial manifestations of a hemolytic process and may be the ones that bring the illness to the attention of a physician. The stones are of the pigment type and are presumed to be a consequence of the continuously excessive bilirubin load presented to the gallbladder. Cholelithiasis has been observed in young children,<sup>38</sup> but is rare prior to puberty.<sup>31</sup> The incidence increases with age; 85% of adults with hereditary spherocytosis are affected.

### Leg Ulcers

Chronic ulcerations of the legs are a peculiar and relatively uncommon complication of chronic hemolytic disease. They are particularly characteristic of hereditary spherocytosis<sup>32,43,51</sup> (Fig. 21-1, page 753) and sickle

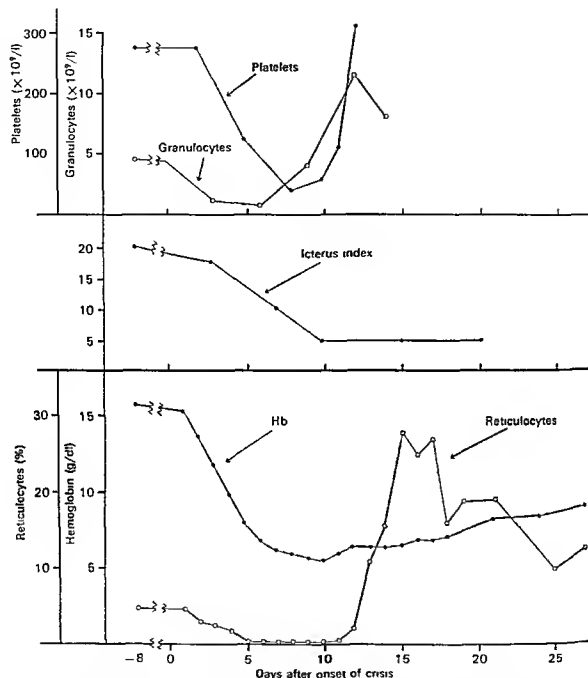


Fig. 20-1 Severe aplastic crisis in a patient with hereditary spherocytosis who previously had well compensated hemolysis. Note the profound reticulocytopenia during the early phases of the reaction, followed by reticulocytosis. As occurred in this patient, jaundice often decreases during the crisis, and mild, transient leukopenia and thrombocytopenia are not unusual. (Plotted from the data of Owen<sup>45</sup>)

cell anemia<sup>35,53</sup> (Fig. 25-6, page 842), but may also be seen in association with other hemolytic disorders.<sup>30,41,50</sup> The ulcers often are bilateral and tend to involve the areas

over the medial or lateral malleoli or those above these prominences. When severe, they may extend a considerable distance up the leg and may completely surround it. They tend



to be chronic or recurrent, and, upon healing, leave indurated and pigmented areas of the skin.<sup>32</sup> Interruption of the hemolytic process, eg, by splenectomy in hereditary spherocytosis, is followed by healing of the ulcers.<sup>43,51</sup>

### ***Skeletal Abnormalities***

When hemolytic anemia is severe during active phases of growth and development, the pronounced expansion of the erythroid bone marrow may lead to a tower-shaped skull, thickening and striation of frontal and parietal bones, maxillary and dental abnormalities, and other distortions of bony structures.<sup>33</sup> Such abnormalities are particularly characteristic of severe thalassemia major and are described fully in the chapter dealing with that illness (page 863). They may also occur in patients with sickle cell anemia (page 832) and in exceptional patients with other forms of congenital hemolytic anemia.<sup>33,50</sup>

### ***Acquired Hemolytic Anemia***

If hemolytic anemia develops *acutely*, eg, after the transfusion of incompatible blood, or the ingestion of an "oxidant" drug by patients with glucose-6-phosphate dehydrogenase deficiency, the symptoms may suggest an acute febrile illness. Some instances of autoimmune hemolytic anemia, thrombotic thrombocytopenic purpura, and other hemolytic disorders may also begin abruptly.<sup>52</sup> Aching pains in the back, abdomen, or limbs are common, as are headaches, malaise, vomiting, shaking chills, and fever.<sup>52</sup> Abdominal pain may be severe, and the accompanying muscular spasm and rigidity may simulate the signs of an acute abdominal condition requiring surgical treatment.<sup>52</sup> Profound prostration and shock may develop, followed by oliguria or anuria (Chapter 11, page 480). Pallor, jaundice, tachycardia, and other symptoms of severe anemia may be prominent.

More often, acquired hemolytic anemia begins *insidiously*. The anemia develops gradually over a period of weeks or months. Cardiovascular adjustments to the anemia

may be quite good and there may be few symptoms. The clinical picture may be similar to that described for congenital hemolytic disease. Pallor, scleral icterus, or a jaundiced complexion may be the first evidence of illness, and often is noticed by friends or associates before it is appreciated by the patient or his family. When anemia is more severe, the patient commonly complains of weakness, fatigability, dyspnea, or other cardiovascular symptoms (page 535). As in congenital hemolytic anemia, the course may be interrupted by aplastic crises.<sup>48</sup>

In other instances, the clinical setting may be dominated by the manifestations of an underlying disease of which the hemolytic anemia is one manifestation. Thus, for example, signs and symptoms of lymphoma, lupus erythematosus, or mycoplasma pneumonia may overshadow those of the associated hemolytic process.

## **Laboratory Manifestations**

Laboratory findings in hemolytic anemia can be divided conveniently into three groups: (1) those related to the increase in erythrocyte destruction, (2) those related to the compensatory increase in the rate of erythropoiesis, and (3) those found only in particular varieties of hemolytic anemia and therefore useful in differential diagnosis.

### ***Signs of Excessive Red Cell Destruction (Table 20-5)***

#### ***Erythrocyte Survival***

The life span of the red cell can be measured by any of the methods described in Chapter 5 (page 195), but the method based on random labeling with <sup>51</sup>chromium has been used much more frequently than the others. As measured by this technique, the half-disappearance time ( $t_{1/2}$  Cr) is nearly always reduced in hemolytic disease, but the degree of reduction varies from one patient to another, even among those with the same disease (Fig. 20-2). On the average,  $t_{1/2}$  Cr is reduced to about half the normal value;

**Table 20-5. Laboratory Signs of Accelerated Red Cell Destruction**

- A Decreased erythrocyte life span
- B Increased catabolism of heme
  - 1 Increased serum unconjugated bilirubin
  - 2 Increased endogenous carbon monoxide production
  - 3 Increased rate of bilirubin production
  - 4 Increased rate of urobilinogen excretion
- C Increased serum lactate dehydrogenase activity
- D Signs of intravascular hemolysis
  - 1 Hemoglobinemia
  - 2 Absence of haptoglobin\*
  - 3 Hemoglobinuria
  - 4 Hemosiderinuria
  - 5 Methemalbuminemia
  - 6 Reduced serum hemopexin  $\curvearrowright$

\*Also occurs in association with predominantly extravascular hemolytic processes

however, because of the nonlinear relation between  $t_{1/2}$  Cr and erythrocyte life span, the reduction in the latter is greater than might be supposed. Thus, for example, a 50% reduction in  $t_{1/2}$  Cr corresponds to an erythrocyte life span of about 30 days, or 25% of normal. The  $t_{1/2}$  tends to be lowest in patients with the greatest degree of anemia, but the correlation is not a very close one.

Erythrocyte life span determinations are time-consuming and expensive. Furthermore, they are rarely necessary because the degree of red cell survival usually can be approximated by analysis of more easily obtained data, such as serial observations of the degree of anemia, reticulocytosis, and jaundice. For these reasons, determination of red cell survival should not be considered a routine procedure in examination of patients suspected of having hemolytic anemia. Instead, it should be reserved for those presenting especially difficult diagnostic problems.

### **Catabolism of Heme**

In hemolytic disease the heme moiety of hemoglobin is catabolized at a greatly accelerated rate, and excretion of the principal heme catabolites—bile pigments and carbon monoxide—is proportionately increased

(Chapter 5). These alterations provide clinically useful signs of hemolysis.

**SERUM BILIRUBIN.** The amount of bilirubin in the circulation depends partly on the rate at which the bilirubin is formed and partly on the efficiency with which it is excreted by the liver. It is not surprising, therefore, that the serum bilirubin level is an unreliable index of the rate of red cell destruction. Occasionally, it falls within the normal range despite brisk hemolytic disease. The total serum bilirubin was normal (less than 1.0 mg/dl) in 25% of 72 patients with hereditary spherocytosis and ranged from 1.0 to 4.8 mg/dl in the remaining 75% (Fig. 20-3).<sup>101</sup> Similarly, it was increased in 55% of 120 patients with immunohemolytic anemia and normal in the remaining 45%.<sup>100</sup> Except during the neonatal period, values greater than 5 mg/dl are unusual in patients with hemolytic anemia and suggest coexisting hepatic dysfunction.

The increased serum bilirubin in hemolysis almost always consists of the unconjugated ("indirect reacting") pigment. The conjugated fraction remains within normal limits, and no bilirubin is found in the urine. A number of other illnesses are associated with unconjugated bilirubinemia (Table 20-6), and these must be distinguished from hemolytic anemia.

**RATE OF HEME CATABOLISM.** Because the serum level of unconjugated bilirubin is a poor index of the rate of heme catabolism, and because it remains within normal limits in a sizable proportion of patients with hemolytic disease, there is need for more sensitive and quantitative techniques. As yet, no such method that is both simple and accurate has been developed. Determinations of the rate of endogenous carbon monoxide production or of bilirubin turnover (page 216) provide accurate assessments of the rate of heme catabolism. With these methods, values of about 2 to 10 times the normal rate have been detected in small groups of patients with hemolytic disease. However, at the present stage of development, these methods are too complex for the routine clinical laboratory.

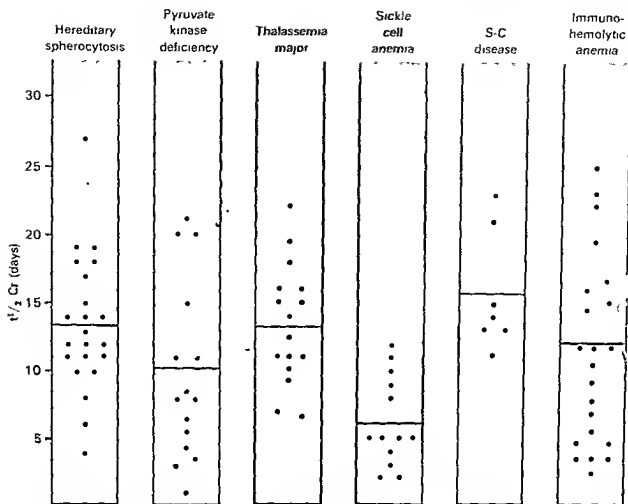


Fig 20-2. Erythrocyte life span ( $t_{1/2}$ ) as measured by  $^{51}\text{Cr}$  tagging of red cells in several hemolytic states. Each dot represents the results in a single patient. The horizontal lines indicate the means and the shaded areas outline the normal range. The data are derived from the examination of 22 patients with hereditary spherocytosis,<sup>61,77,84,112,117</sup> 15 with pyruvate kinase deficiency,<sup>30, 55, 65, 74, 89, 92, 104, 104, 109, 120</sup> 16 with thalassemia major,<sup>83, 125</sup> 13 with sickle cell anemia,<sup>121</sup> 7 with S-C disease<sup>121</sup> and 22 with immuno-hemolytic anemia.<sup>75, 117</sup>

Quantitative measurements of fecal urobilinogen excretion (page 217) are more available. They provide a more sensitive index of hemolysis than does the serum bilirubin level; however, they have the undesirable feature of requiring the accurate collection and processing of timed fecal specimens. Miller et al found that if the excretion of urobilinogen was expressed in relation to the estimated circulating hemoglobin (Hb) mass (the "hemolytic index"), the normal value was 11 to 21 mg/day/100 g Hb.<sup>102</sup> In 12 patients with hemolytic disease, values ranged from 32 to 792 mg/day/100 g Hb and averaged 255 mg/day/100 g Hb. Since formation of

urobilinogen depends upon intestinal bacteria (page 213), falsely low values are to be expected in patients receiving broad-spectrum antibiotics.

### Lactate Dehydrogenase

Serum lactate dehydrogenase (LDH) activity often is increased in hemolytic anemia, although not to as great an extent as in megaloblastic anemia (see Fig. 14-6, page 573). In seven patients with various types of hemolytic anemia, serum LDH averaged 580 U/ml and ranged from 285 to 1160 U/ml<sup>82</sup> as compared with the upper limit of normal

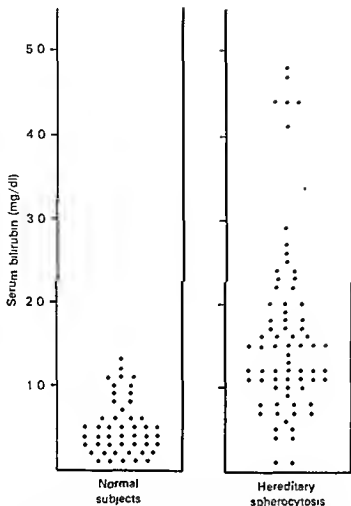


Fig 20-3 Total serum bilirubin values in 48 normal subjects and 72 patients with hereditary spherocytosis (From the data of MacKinney et al.<sup>101</sup>)

of 240 U/ml. If LDH isozymes are determined, LDH-2 predominates in hemolytic anemia, whereas LDH-1 predominates in megaloblastic conditions.<sup>127</sup> The increase in LDH is thought to result from liberation of the erythrocyte enzyme into the plasma during hemolysis.<sup>82</sup>

#### *Signs of Intravascular Hemolysis*

When erythrocytes are destroyed within the circulation, and also when extravascular destruction is so rapid that the capacity of the reticuloendothelial system is exceeded, hemoglobin is released into the plasma. The

hemoglobin and its heme group are disposed of by several mechanisms (page 205), and characteristic laboratory abnormalities are found (Table 20-5, D).

**HEMOGLOBINEMIA.** At low concentrations, plasma hemoglobin may be measured by means of the benzidine reaction,<sup>90,124</sup> which detects not only hemoglobin but also any other heme pigments that may be present. If special precautions are observed to avoid artifactual hemolysis during collection of blood, normally values of less than 0.001 g/dl of plasma are found. Plasma usually appears visibly red when hemoglobin exceeds

**Table 20-6. Conditions Associated with Increased Levels of Unconjugated Bilirubin\***

- |   |   |
|---|---|
| A | Excessive heme catabolism   |
|   | 1 Hemolytic anemia  |
|   | 2 Ineffective erythropoiesis  |
|   | 3 Extravasation of blood into tissues or body cavities                    |
| B | Defective bilirubin conjugation   |
|   | 1 Complete deficiency of glucuronyl transferase (Crigler-Najjar syndrome) |
|   | 2 Partial deficiency of glucuronyl transferase                            |
|   | 3 Inhibition by certain steroids (eg, pregnanediol)                       |
|   | a Lucey-Driscoll syndrome (transient familial hyperbilirubinemia)         |
|   | b Breast-milk jaundice  |
|   | c Pregnancy   |
| C | Mixed pathogenesis (decreased uptake and defective conjugation)           |
|   | 1 Neonatal hyperbilirubinemia   |
|   | 2 Drug-induced hyperbilirubinemia   |
|   | a Flavaspidic acid  |
|   | b Novobiocin  |
|   | c Synkevite   |
|   | d Estrogens   |
| D | Of uncertain pathogenesis   |
|   | 1 Gilbert's syndrome  |
|   | 2 (?) Associated with some cases of hepatitis                             |
|   | 3 Portacaval shunt hyperbilirubinemia                                     |
|   | 4 High altitude exposure  |
|   | 5 Fasting   |
|   | 6 Exercise  |

\*Modified from LW Powell.<sup>110</sup>

0.05 g/dl. At levels greater than 0.1 g/dl, hemoglobin can be measured directly by the cyanomethemoglobin method (page 114).

Plasma hemoglobin levels were found to be normal in patients with most hereditary hemolytic anemias, including hereditary spherocytosis, but values of 0.015 to 0.06 g/dl were noted in patients with sickle cell anemia and thalassemia major.<sup>76</sup> Levels also were increased in severe, acquired, immunohemolytic anemia, at times reaching 0.1 g/dl.<sup>76</sup> Particularly high values, up to 1.0 g/dl, are found only in patients with disorders associated with predominantly intravascular hemolysis.<sup>88</sup>

**ABSENCE OF HAPTOGLOBIN.** When hemoglobin (Hb) first appears in plasma, it becomes bound to haptoglobin (Hp) and the

HpHb complex is removed by the hepatocyte (page 207). Since there is no compensatory increase in Hp synthesis, Hp tends to disappear from the plasma in hemolytic diseases. Plasma haptoglobins may be measured by methods based on the peroxidase activity of the HpHb complex<sup>106</sup> or by electrophoretic<sup>123</sup> or chromatographic<sup>100</sup> measurement of hemoglobin binding capacity.

Absence of haptoglobin is one of the most sensitive signs of hemolysis; it is so sensitive, in fact, that it occurs even in hemolytic diseases that are not associated with hemoglobinemia, such as hereditary spherocytosis,<sup>70</sup> hereditary elliptocytosis,<sup>70</sup> and pyruvate kinase deficiency.<sup>50</sup> In most patients, haptoglobin was found to be absent whenever red cell destruction exceeded twice the normal rate, regardless of diagnosis.<sup>70</sup> However, haptoglobin levels increase in response to infections, malignant disease, or steroid therapy, and when any of these conditions complicated the hemolytic state, haptoglobin was demonstrable.

**HEMOGLOBINURIA.** If plasma hemoglobin exceeds the haptoglobin binding capacity, hemoglobin dimers are excreted into the urine, resulting in hemoglobinuria. Urine that contains hemoglobin ranges in color from faint pink to deeper red, or even to an almost black color similar to that of a "cola" beverage. The color depends on the concentration of hemoglobin as well as on the oxidation state and degree of dissociation of the heme group.

Hemoglobinuria can be distinguished from hematuria (whole red blood cells in the urine) by microscopic examination of a freshly voided urine specimen. Urine also may be red because of certain drugs (pyridium, antipyrine) or food (beets) taken by the patient, or because of porphyrinuria (Chapter 32) or myoglobinuria. Of these various red urinary pigments, only hemoglobin and myoglobin produce a positive reaction in the commonly available tests for occult blood, which are based on the benzidine or orthotoluidine<sup>69</sup> reactions (Occultest or Hemastix, Ames Laboratories, Elkhart, Indiana.)

Hemoglobinuria must be distinguished from *myoglobinuria*, which occurs as the result of massive muscle injury by trauma,<sup>71</sup> electric shock, arterial thrombosis,<sup>72</sup> certain toxins ("haft" disease),<sup>63 90, 122</sup> Malayan sea-snake-bite,<sup>113</sup> "idiopathic" myoglobinuria,<sup>116</sup> myophosphorylase deficiency (McArdle's disease),<sup>62 118</sup> and other causes.<sup>116</sup> Myoglobin is a heme pigment of small molecular weight (17,000); it is not bound by haptoglobin and therefore does not accumulate to an appreciable extent in plasma. Thus, inspection of the plasma can help to distinguish myoglobinuria from hemoglobinuria, the presence of a red color favoring the latter. More precise identification is accomplished by electrophoresis on paper, on starch or acrylamide gels or by spectrophotometry. The last is facilitated by treatment with carbon monoxide since there is greater divergence between the bands of carboxyhemoglobin and carboxymyoglobin than between the oxygenated forms. Unlike hemoglobin, myoglobin remains in solution after 80% saturation of the urine with ammonium sulfate.<sup>67</sup>

### Urine Iron Excretion

Hemoglobin in the glomerular filtrate is partially reabsorbed by the proximal tubular cells, and the hemoglobin iron is incorporated into ferritin and hemosiderin. Subsequently, the iron-containing tubular cells are sloughed into the urine. Hemosiderinuria and increased urinary iron excretion, therefore, constitute reliable evidence that hemoglobinemia has occurred in the recent past.<sup>76 88</sup> However, following an acute episode of intravascular hemolysis, it may be several days before increased iron excretion can be detected, and the abnormality may persist for some time after the episode has terminated. In most conditions associated with chronic intravascular hemolysis, increased iron excretion is a constant finding whereas hemoglobinuria occurs only intermittently. The only non-hemolytic disorder in which increased iron excretion is found is hemochromatosis.<sup>87</sup>

*Hemosiderinuria* may be detected by means of a qualitative test based on the Prussian

blue reaction.<sup>70</sup> Alternatively, urinary iron may be determined spectrophotometrically after wet digestion of a measured urine specimen.<sup>114, 119</sup> The normal value for urinary iron excretion is less than 0.1 mg/day. In a variety of disorders associated with intravascular hemolysis, urinary iron was increased to between 3 and 11 mg/day.<sup>119</sup> Normal or nearly normal values were found in pernicious anemia and in hereditary spherocytosis. In nine patients with hemolysis from prosthetic cardiac valves, urinary iron was increased to 0.8 and even 10.8 mg/day.<sup>114</sup> After correction of the abnormality by insertion of a heterograft, urinary iron decreased exponentially with time, but did not reach normal levels until after 6 to 10 months.

It appears, therefore, that urinary iron determination may be a useful and consistent sign of intravascular hemolysis. However, it may not accurately reflect the current clinical situation because of its persistence after the process has terminated.

### Methemalbumin and Hemopexin

Hemoglobin in plasma is readily oxidized to methemoglobin, from which the heme group detaches relatively easily. The liberated heme becomes bound to hemopexin and also to albumin, forming methemalbumin (page 208). Hemopexin-heme and methemalbumin impart a coffee-brown color to plasma. With either, a spectral absorption band is observed at 620 to 630 nm, which, unlike a similar band in methemoglobin, will not disappear if hydrogen peroxide is added. Upon the addition of ammonium sulfide, the 620 to 630 nm band disappears and a band at 558 nm is formed (Schumm's test).<sup>115</sup>

The presence of these pigments suggests intravascular hemolysis.<sup>85</sup> They have also been observed in association with hemorrhagic pancreatitis.<sup>97</sup> Like haptoglobin, hemopexin can be depleted in the course of serving its function,<sup>103</sup> but this occurs much less regularly than with haptoglobin. Subnormal serum hemopexin values have been noted particularly in patients with thalassemia major or sickle cell anemia.<sup>103</sup>

### Signs of Accelerated Erythropoiesis

The laboratory measures relating to increased erythropoiesis are listed in Table 20-7. In general, these findings are detected in chronic hemolytic disease, and will appear at different time intervals following an acute hemolytic episode. They also occur following hemorrhage and after specific therapy for anemia due to iron, folate or vitamin B<sub>12</sub> deficiency. It is sometimes useful to divide the signs of accelerated erythropoiesis into those that reflect *total* erythropoiesis (eg, the degree of erythroid hyperplasia and the plasma iron transport rate) and those that reflect *effective* erythropoiesis (eg, the reticulocyte count and the erythrocyte iron turnover rate). In conditions associated with ineffective erythropoiesis (page 550), measures of total erythropoiesis are increased, but measures of effective erythropoiesis are not.

#### Reticulocytosis

An increase in circulating reticulocytes is among the simplest and most reliable signs of accelerated erythrocyte production. Reticulocytes are young red cells containing ribosomes (page 83). Methods for detecting and enumerating them have been discussed in Chapter 3 (page 119). For clinical purposes, reticulocytes most often are reported as a percentage of the number of erythrocytes in the sample evaluated. Expressed in this way, the normal range is 0.8 to 2.5% in men and 0.8 to 4.1% in women.<sup>60</sup> Theoretically,

this percentage can increase either because there are more reticulocytes in the circulation or because there are fewer mature cells. For this reason, some prefer to "correct" for anemia by multiplying the percentage by a ratio of the patient's VPRC (or hemoglobin) to an average normal value. Thus, the "corrected" reticulocyte count equals:

$$\text{Reticulocytes (\%)} \times \frac{\text{Patient's VPRC (1/l)}}{0.45}$$

Alternatively, it is possible to express reticulocyte counts in absolute numbers (reticulocytes per liter) by multiplying the red cell count by the reticulocyte percentage. In these terms, the average normal value is about  $90 \times 10^9/l$ .

However, even these corrected counts are not perfect indices of production, for the percentage of reticulocytes can also be altered by premature release from the marrow ("shift"). A *reticulocyte production index* (RPI) has been proposed to correct both for "shift" and for anemia.<sup>93</sup> It is calculated according to the formula:

$$\text{RPI} = \frac{\text{Reticulocyte percentage}}{\text{Reticulocyte maturation time (days)}} \times \frac{\text{Patient's VPRC (1/l)}}{0.45}$$

The degree of "shift" is related to the intensity of stimulation by erythropoietin. Thus, the maturation time of the reticulocyte (in the circulation) is taken to be 1.0 day at a VPRC of 0.45 l/l, 1.5 days at 0.35, 2.0 days at 0.25, and 2.5 days at 0.15.

Example: VPRC = 0.25 l/l, reticulocytes = 20%

$$\text{RPI} = \frac{20}{2.0} \times \frac{0.25}{0.45} = 5.5,$$

indicating that erythrocyte production is increased to 5.5 times the normal rate. Although this mode of expression probably is a more accurate reflection of the erythrocyte production rate, it has not been demonstrated that diagnostic precision is improved thereby.

In most varieties of hemolytic anemia, the reticulocyte count is consistently increased to levels that correlate fairly well with the severity of the process (Table 20-8). Exceptions

Table 20-7. Laboratory Signs of Accelerated Erythropoiesis

A Blood	
1	Reticulocytosis (polychromatophilia, stippling)
2	Macrocytosis
3	Erythroblastosis
4	Leukocytosis and thrombocytosis
B Bone marrow	
1	Erythroid hyperplasia
C Ferroketic	
1	Increased plasma iron turnover (PIT)
2	Increased erythrocyte iron turnover (EIT)

**Table 20-8. Reticulocytes and Mean Corpuscular Volume (MCV) in Several Varieties of Hemolytic Anemia**

Condition	Number of Cases	Reticulocytes* (%)	MCV* (fl)
Normal	—	16 ± 0.5	90 ± 3
Hereditary spherocytosis <sup>101</sup>	76	9.9 ± 4.9	83 ± 9.5
Pyruvate kinase deficiency <sup>59,77</sup>	9	31.3 ± 25.0†	115 ± 19
Sickle cell anemia <sup>77</sup>	—	17.0 ± 5.0	81 ± 10
Paroxysmal nocturnal hemoglobinuria <sup>77</sup>	12	22.0 ± 11.0	118 ± 14
Immunohemolytic anemia <sup>77</sup>	12	28.4 ± 12.0	111 ± 11

\*Values are means ± 1SD  
 †Highest values occur following splenectomy

occur chiefly during aplastic crises. Thus, of 76 patients with hereditary spherocytosis, the reticulocyte count was normal in only one and less than 5% in only 8.<sup>101</sup> In contrast, in idiopathic immunohemolytic anemia a larger proportion of patients were found to have normal reticulocyte counts.<sup>109</sup> Values of less than 2% were found in 26% of 35 such patients. In these subjects, the picture appeared to be complicated by generalized hypoplasia or erythroid hypoplasia of the marrow, suggesting that the autoantibodies were directed against marrow elements as well as circulating erythrocytes.

When reticulocytes are increased, *polychromatophilia* and *fine stippling* (page 83) may be found on routinely stained smears of blood. Since reticulocytes, especially those that are prematurely released from a stimulated marrow, tend to be larger than normal cells, *macrocytosis* usually accompanies reticulocytosis (Table 20-8). Exceptions occur in hereditary spherocytosis and sickle cell anemia, diseases in which the intrinsic defect of the cell tends to decrease its size.

*Other Morphologic Findings in the Blood*

When hemolysis is brisk, nucleated erythrocytes may be found in the blood (*erythroblastosis*). Usually, these amount to less than 1% of all the nucleated cells in the blood. In infants, however, erythroblastosis may be much more striking, especially in hemolytic disease of the newborn (page 899).

*Neutrophilic leukocytosis and thrombocytosis* may accompany hemolytic anemia. These findings tend to be most common and most pronounced in acute hemolytic anemias. Leukocyte counts as high as  $132 \times 10^9/l$  have been recorded under such circumstances. Platelets are not only numerous, but also large in size. The changes are less pronounced in chronic hemolytic processes. However, leukopenia and thrombocytopenia are unusual findings in hemolytic disease. Their presence suggests such diagnoses as paroxysmal nocturnal hemoglobinuria, hemolysis accompanying systemic lupus erythematosus, or, in a minority of cases, idiopathic autoimmune hemolytic anemia.

*Bone Marrow*

The major alteration in the bone marrow in hemolytic anemia is *erythroid hyperplasia*. Although methods have been developed for quantitating the marrow erythroid mass<sup>91</sup> (page 61), these are not practical for routine clinical use. Consequently, erythroid hyperplasia must be detected by examination of marrow aspirates or biopsy specimens. Marrow examination should be considered a qualitative or semiquantitative technique, and is not suitable for precise calculation of production rates. It is important to remember that marrow specimens represent only a tiny sample of the marrow and may not always accurately reflect the degree of change.

In smears of aspirated specimens, erythroid



hyperplasia is manifested by a reduction in the M:E ratio (page 71). In hemolytic disease, the ratio usually is less than 1.5 and may be as low as 0.5. However, since the M:E ratio also may be decreased as the result of reduction in granulocyte precursors, the meaning of the ratio must be evaluated in relation to the total clinical picture. It is hazardous to judge the cellularity of the marrow from the cellularity of an aspirated specimen. Marrow *biopsy specimens* should be used for this purpose, and with them more accurate qualitative judgments regarding the presence of erythroid hyperplasia can be made.

#### *Ferrokinetic Studies (Chapter 4)*

The plasma iron transport rate (PITR) is considered to be a measure of total erythropoiesis and correlates well with the degree of erythroid hyperplasia.<sup>86,91</sup> The erythrocyte iron turnover rate (EITR) is a measure of effective erythropoiesis and correlates well with the reticulocyte production index (page 165). In hemolytic anemia, the PITR averages two to five times the normal rate, and the EITR is increased two- to four-fold<sup>86</sup> (Table 20-9). These ferrokinetic measures provide accurate information regarding rates of erythropoiesis. Nevertheless, they are

unnecessary in the overwhelming majority of patients because the reticulocyte count and other easily obtained determinations are simpler, faster, considerably less expensive, and nearly as accurate.

#### *Laboratory Findings Useful in Differential Diagnosis*

##### *Specific Morphologic Abnormalities*

A competent, careful examination of a well-prepared blood smear is the single most valuable procedure in defining the underlying disorder leading to hemolytic anemia. Detection of certain distortions of red cell shape is of particular diagnostic utility because their presence suggests only one or a few entities. Descriptive features of such poikilocytes have been presented in Chapter 13 (page 540). Their major characteristics are summarized in Table 13-3, and they are illustrated in Figure 13-2.

Spherical erythrocytes, or *spherocytes*, are the hallmark of hereditary spherocytosis (Chapter 21). They are also found in most patients with acquired immunohemolytic anemia (Chapter 27), thermal injury, hypophosphatemia, or certain kinds of chemical poisoning. Spiculated cells, or *acanthocytes*, indicate disturbed erythrocyte lipid com-

**Table 20-9. Ferrokinetic Measurements in Various Hemolytic Anemias (Mean Values)\***

Condition	Number of Patients	VPRC (l/l)	Plasma FE (μg/dl)	PITR (mg/dl/day)	EIT (mg/dl/day)
Normal	107	0.45	105	0.7	0.56
Hereditary Spherocytosis	15	0.35	118	3.5	2.0
Non-Spherocytic Hemolytic Anemia	6	0.37	110	2.5	—
Sickle Cell Anemia	20	0.24	118	3.1	1.9
Hb S-C Disease	7	0.33	99	1.6	1.2
Immunohemolytic Anemia	10	0.29	116	2.3	1.8
Paroxysmal Nocturnal Hemoglobinuria	11	0.29	120	2.0	1.3

Abbreviations: VPRC, volume packed red cells; Fe, iron; PITR, plasma iron transport rate; EIT, erythrocyte iron turnover.

\*Data from CA Finch et al.<sup>84</sup>

position; they occur in association with abetalipoproteinemia (Chapter 21) and the "spur-cell anemia" that occasionally accompanies hepatic cirrhosis (page 708). *Stomatocytes*, which suggest a disturbance in red cell cation content, are found in association with a rare inherited hemolytic disease (Chapter 21) and also occur as a transient abnormality in acute alcoholics. *Target cells* are characteristic of thalassemia (Chapter 26), the homozygous abnormal hemoglobin states (Chapter 25), and lecithin-cholesterol acyl transferase (LCAT) deficiency (Chapter 21); they also occur in nonhemolytic states, such as obstructive jaundice and following splenectomy. Oval cells or *elliptocytes* are the sine qua non of hereditary elliptocytosis (Chapter 21). *Sickle cell anemia* was named after the unmistakable sickle-shaped poikilocytes that characterize that illness (Chapter 25). Finally, large numbers of *schistocytes*, *helmet cells*, or other fragmented red cells suggest hemolysis associated with physical trauma to the erythrocyte or with diseases affecting small blood vessels (Chapter 28).

*Erythrophagocytosis* (page 202), or the presence in blood of phagocytic cells containing recognizable whole red cells, is an

uncommon finding. When detected, it suggests damage to the red cell surface, especially that induced by complement-fixing antibodies, but also by protozoan and bacterial infectious agents and certain chemical poisons.

*Autoagglutination* may be apparent in blood smears or even visible to the naked eye when the blood is allowed to flow along the side of a glass container. The phenomenon is particularly characteristic of immunohemolytic disease due to cold agglutinins (page 921). Autoagglutination must be distinguished from rouleau formation (page 1611), a manifestation of myeloma and related diseases and the phenomenon responsible for accelerated rates of erythrocyte sedimentation (page 126).

### The Osmotic (Hypotonic Saline) Fragility Test

The osmotic fragility test is a measure of the resistance of erythrocytes to hemolysis by osmotic stress. The test consists of exposing red cells to decreasing strengths of hypotonic saline solutions and measuring the degree of hemolysis. Many techniques have been described,<sup>81,107</sup> including a semiautomated method.<sup>79</sup> To construct the conventional osmotic fragility curve, percent hemolysis is plotted on the vertical axis against decreasing saline concentration on the horizontal axis. A symmetrical curve, sigmoidal in shape, is obtained in most subjects (Fig. 20-4). Alternatively, the data can be plotted on probability paper<sup>107</sup> or in the form of incremental hemolysis curves.<sup>81</sup>

Osmotic fragility can also be described in terms of the saline concentration at which hemolysis begins (normally, 0.45 to 0.50 g/dl) or at which it is complete (normally 0.30 to 0.33 g/dl). Especially useful is the median corpuscular fragility (MCF), the saline concentration at which 50% of the cells hemolyze (normally 0.40 to 0.455 g/dl). (See Appendix A for additional normal values.) Increased fragility is indicated by a shift of the curve to the left (Fig. 20-4, B) or a high value for MCF. Osmotic resistance (reduced

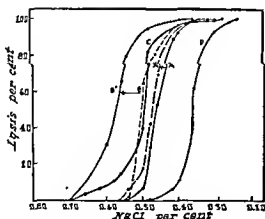


Fig 20-4 Normal and abnormal osmotic fragility curves. A, Normal curve. A', the moderate increase in osmotic fragility on incubation. B, Hereditary spherocytosis. B', greatly increased osmotic fragility following incubation. C, Acquired hemolytic anemia. D, Increased resistance of red corpuscles from a patient with thalassemia minor.

fragility) is signified by a rightward shift (Fig. 20-4,D) and a reduced MCF value.

Increased osmotic fragility is observed in conditions associated with spherocytosis (page 733). This may be because very little fluid needs to be absorbed by cells of spherical shape to bring them to the point at which their membrane is stretched to the bursting point (Fig. 21-4), but other factors such as "leakiness" of the membrane (increased permeability to cations) and the intracellular osmotic pressure may also be important (page 757). With prior incubation of sterile blood for 24 hours,<sup>20</sup> the increased osmotic fragility of spherocytes is greatly accentuated (Fig. 20-4, B-B'), whereas normal cells become only slightly more fragile (Fig. 20-4, A-A'). The osmotic fragility of unincubated blood may, in fact, be normal in some patients with hereditary spherocytosis, and incubation of the red corpuscles may be necessary to distinguish them from normal.

Increased resistance to breakdown in hypotonic saline solutions is observed in thalassemia, sickle cell anemia, and other disorders in which many leptocytes, including "target" cells, are found (Fig. 20-4, D). Sometimes a small proportion of unusually fragile cells may also be detectable in these conditions. With prior incubation, target cells and leptocytes may become even more resistant to osmotic stress and there is a further rightward shift of the curve.

Determination of osmotic fragility thus appears to be chiefly of value in confirming important morphologic findings, especially the presence of spherocytes but also that of leptocytes. It is unusual to find that the osmotic fragility test provides information that was not already available from an expert examination of a well-prepared, stained blood smear.

### **Mechanical Fragility**

The sensitivity of erythrocytes to physical trauma has been measured by shaking the cells in a flask with glass beads<sup>120</sup> or by traumatizing them in other ways.<sup>60</sup> The method must be carefully standardized if reproduc-

ble results are to be obtained. In general, mechanical fragility is increased in the presence of spherocytes, sickled cells, or agglutinated cells, but not with other types of poikilocytes.<sup>120</sup> The test has not been widely applied, since it provides little or no information that cannot be obtained by morphologic means or with the osmotic fragility test.

### **Autohemolysis**

The test for autohemolysis<sup>20,98,111</sup> depends on the incubation of sterile, defibrinated blood at 37° C for 48 hours and measurement of the amount of spontaneous hemolysis that occurs. Comparison is made with the amount of hemolysis occurring when glucose or ATP has been added prior to incubation. With normal blood, little hemolysis takes place in 48 hours and even less when glucose or ATP is added (Table 20-10). On the basis of the degree of autohemolysis and the correction by ATP and glucose, Selwyn and Dacie<sup>20</sup> defined two abnormal patterns, Type I and Type II (Table 20-10). With the Type I pattern, autohemolysis is only slightly to moderately increased and there is incomplete correction with either ATP or glucose. With the Type II pattern, autohemolysis is greatly increased and glucose has no effect, but ATP restores the value to normal. A third pattern was found in hereditary spherocytosis, namely, greatly increased autohemolysis with complete or nearly complete correction with either ATP or glucose.

Subsequent studies indicated that Type II autohemolysis is characteristic of pyruvate kinase (PK) deficiency<sup>50</sup> and that the Type I pattern occurs in glucose-6-phosphate dehydrogenase deficiency. However, the patterns are neither very sensitive nor completely specific. Thus, normal autohemolysis was found in some patients with mild PK deficiency and a Type I pattern in others.<sup>50</sup> Patterns characteristic of various enzyme deficiencies<sup>78,84 98,111</sup> are given in the footnote to Table 20-10.

The autohemolysis test now largely is of historic interest. The tediousness of the method, its lack of specificity and sensitivity,

Table 20-10. The Autohemolysis Test in Various Disorders<sup>73</sup>

Condition†	Autohemolysis (%/48 hr)†		
	No Additive	With Glucose	With ATP
Normal	2.0 (0.2-4.0)	0.3 (0.1-0.6)	0.2 (0.1-0.8)
Type I	3.0 (1-6)	1.3 (0.5-4.0)	1.0 (0.4-2.0)
Type II	13 (8-44)	15 (4-48)	1.0 (0.2-2.0)
Hereditary spherocytosis	16 (6-30)	3 (0.2-14)	3 (1-6)

†Values are means with approximate range in parentheses.

†Type I autohemolysis is typical of hereditary elliptocytosis, unstable hemoglobin disease, and deficiencies of glucose 6-phosphate dehydrogenase, glucose-phosphate isomerase, phosphoglycerate kinase, 2,3-diphosphoglycerate mutase, and 6-phosphogluconate dehydrogenase. Type II autohemolysis is found particularly in pyruvate kinase deficiency but also has been reported in deficiencies of hexokinase, ribose pyrophosphokinase, and 2,3-diphosphoglycerate mutase. A pattern similar to that of hereditary spherocytosis was found in triose phosphate isomerase deficiency.

and the availability of simplified spot tests for specific enzyme deficiencies<sup>85</sup> have greatly limited its value. It still has some utility as a screening test when enzymatic assays are not available.

#### Tests for Hemolytic Disorders Associated with Heinz-Body Formation

In certain disorders the hemolytic process depends on precipitation of hemoglobin, with the formation of inclusions known as Heinz bodies. These inclusions are rapidly removed by the spleen. Heinz-body formation is the principal mechanism of hemolysis in glucose-6-phosphate dehydrogenase (G6PD) deficiency and related disorders (Chapter 23), in unstable hemoglobin disease (Chapter 24), in the thalassemias (Chapter 26), and in certain kinds of chemical injury (page 743). Heinz bodies usually are not observed when ordinary staining procedures are employed, but require special supravital stains (page 29). Cells containing these inclusions may

be found in the blood during an acute drug reaction in subjects with G6PD deficiency and also in splenectomized individuals with unstable hemoglobin disease. However, when the spleen is intact, the inclusions are removed with such efficiency that cells containing them are rarely seen.

It is possible to induce Heinz-body formation in vitro by incubating erythrocytes with acetylphenylhydrazine.<sup>66</sup> The number, size, and rate of formation of the inclusions tend to be greater in cells from patients with the disorders listed above than in other individuals. However, the frequent occurrence of falsely positive results in many anemias has limited the usefulness of this procedure.<sup>84</sup>

The glutathione stability test<sup>64</sup> depends on the observation that, when sensitive cells are exposed to acetylphenylhydrazine, the glutathione level falls rapidly to low levels, whereas the rate and amount of fall are much less in normal cells. The test is technically difficult and infrequently used.

The ascorbate-cyanide test is a sensitive measure of abnormal susceptibility of hemoglobin to peroxidative denaturation.<sup>95</sup> It depends upon the coupled oxidation of ascorbate with oxyhemoglobin to produce hydrogen peroxide. When catalase is inhibited with cyanide, the glutathione-dependent system is required to protect peroxidative damage to hemoglobin. Two ml of whole blood are well-oxygenated to a bright-red color by swirling in air or oxygen. The blood is added to tubes containing 10 mg ascorbate, 5 mg glucose, and 2 drops 0.1 M cyanide, and the mixture is incubated at 37° C with agitation. Normal blood remains red, but in abnormal blood a dark-brown color is observed within two hours.

The test is quickly and easily performed and its reaction is strongly positive in deficiencies of G6PD or glutathione peroxidase and in some unstable hemoglobin disorders.<sup>84</sup> Lesser degrees of darkening were reported in deficiencies of glutathione reductase or pyruvate kinase and in individuals heterozygous for G6PD deficiency.

None of these screening procedures is entirely satisfactory, but the ascorbate-cyanide

test is preferable for routine use because it is so simple. More specific tests, such as enzyme spot tests<sup>65</sup> or the heat stability test for unstable hemoglobins (page 808), also are useful in the differential diagnosis of these disorders.

### Other Tests

Other important laboratory procedures for detecting and differentiating the hemolytic anemias are discussed in chapters dealing with specific entities. Thus, methods for identifying enzyme deficiencies<sup>65</sup> are discussed in Chapters 22 and 23. Those for detecting and defining abnormal hemoglobins appear in Chapter 24; the Coombs' test and other serologic techniques for evaluating immunohemolytic anemias are described in Chapter 27; and tests for paroxysmal nocturnal hemoglobinuria appear in Chapter 29.

## Diagnostic Approach

A final diagnosis of one of the hemolytic anemias is established by a process consisting of two distinct steps: first, demonstrating that a hemolytic anemia is present and, second, determining the specific cause of the condition. A common error made in clinical practice is the pursuit of the second objective before the first has been accomplished. This can be unproductive and wasteful in respect to time and money.

### *Establishing That Hemolytic Anemia Is Present*

The diagnosis of hemolytic anemia is essentially based on kinetic considerations. It requires a careful analysis of data that pertain to rates of red cell production (Table 20-7) and destruction (Table 20-5). In general, no single finding establishes the diagnosis; rather, it depends on a characteristic combination of findings, interpreted in the light of information from a carefully taken history. The presence of hemolytic anemia may be considered established by any of the following:

1. *Evidence of excessive red blood cell destruction and excessive red cell production occurring at the same time.* Perhaps the most common example of this type of evidence is the association of anemia, reticulocytosis, and unconjugated (indirect) hyperbilirubinemia together with historical information excluding other possible causes of these findings. Occult hemorrhage into an organ or a tissue space may simulate this picture, but only transiently (page 698).
2. *Evidence of persistent anemia despite increased erythropoiesis, in the absence of blood loss.* In situations of this type, anemia and reticulocytosis are observed, but jaundice is lacking. Since hemorrhagic anemia and the early stages of response to specific therapy of nutritional anemia may also produce this set of findings, efforts to exclude them from consideration are appropriate. In addition, a quantitative measure of heme catabolism, such as fecal urobilinogen excretion, may be useful in this setting. Still another practical approach to this problem is to make serial observations; if the anemia and reticulocytosis persist without evidence of continuing blood loss, hemolytic anemia is the only tenable diagnosis.
3. *Development of anemia at a rate exceeding that which can be accounted for by a total arrest of erythropoiesis.* A fall in blood hemoglobin concentration at a rate of 1.0 g/dl per week or faster implies hemolysis, blood loss, or rapid hemodilution. If the possibility of the last two can be excluded, the presence of hemolytic anemia is confirmed. This type of presentation might occur during an aplastic crisis in a patient with previously well-compensated disease (Fig. 20-1) or in one having an acute hemolytic reaction. The characteristic reticulocyte response may be suppressed or delayed under such circumstances, and jaundice may or may not be present.
4. *The occurrence of hemoglobinuria or other signs of intravascular blood destruction.* Such episodes may or may not be associated with other signs of hemolysis, de-

**Table 20-11. Conditions Sometimes Mistaken for Hemolytic Anemia**

A	Associated with anemia and reticulocytosis
1	Hemorrhage
2	Recovery from deficiency of iron, folate, or vitamin B <sub>12</sub>
B	Associated with anemia and acholuric jaundice
1	Ineffective erythropoiesis
2	Loss of blood into a body cavity or tissue
C	Acholuric jaundice without anemia (see Table 20-6)
D	Marrow invasion (myelofibrosis metastatic disease)
E	Myoglobinuria

pending on the amount of blood destroyed and the duration of the process. It is important to be certain that the urine pigment is, in fact, hemoglobin and not some other pigment (page 729).

#### *Conditions Sometimes Mistaken for Hemolytic Anemia (Table 20-11)*

As previously mentioned, the anemias due to acute hemorrhage and those due to partially treated deficiency states are characterized by transient anemia and reticulocytosis. They can usually be distinguished from hemolytic disease by the absence of icterus and by a rising VPRC on subsequent determinations.

Anemias caused by ineffective erythropoiesis often are accompanied by acholuric jaundice and by erythroid hyperplasia of the marrow; however, in these the reticulocyte count usually is not increased. In equivocal situations it may be necessary to measure erythrocyte survival, which will be distinctly shortened in hemolytic disease and normal or nearly so in association with ineffective erythropoiesis. The two conditions can also be distinguished by means of ferrokinetic studies (page 167).

A particularly confusing situation may arise following occult hemorrhage into the retroperitoneal space or other tissue compartments. When such hemorrhage occurs, anemia develops rapidly and reticulocytosis follows. Furthermore, indirect hyperbilirubinemia may occur as the result of reabsorption of the products of hemoglobin breakdown at

the site of hemorrhage. Thus, the picture of hemolytic anemia may be simulated in several ways. Diagnosis depends on detecting signs of the hemorrhage itself or of the disease process leading to it. If occult hemorrhage is suspected, serial observations usually clarify the situation, for, once the hemorrhage has ceased, the VPRC, reticulocyte count, and bilirubin values will return to normal.

In individuals with acholuric jaundice but without anemia, the differential diagnosis lies between a compensated hemolytic state and Gilbert's syndrome or other disorders of bilirubin catabolism (Table 20-6). It is usual to detect reticulocytosis or morphologic abnormalities of erythrocytes in the former. However, in mild compensated hemolytic disease, one cannot always be certain that the blood will be abnormal. Fasting induces an exaggerated increase in bilirubin levels in Gilbert's syndrome,<sup>110</sup> but the usefulness of this procedure in distinguishing Gilbert's syndrome from hemolytic anemia remains to be established. If the distinction is considered important, erythrocyte survival may be measured. However, if the disease is asymptomatic there may be little to gain from precise diagnosis.

Anemia associated with "marrow invasion" (Chapter 57) may be accompanied by erythroblastosis and bizarre abnormalities of erythrocyte shape. Mild reticulocytosis may develop because of premature release ("shift," page 731). There usually is no jaundice, however, and evidence of the invasive disease may be detected by examination of the bone marrow.

The differentiation of myoglobinuria from hemoglobinuria was discussed on page 729.

#### *Determining the Specific Cause*

The diagnostic analysis should begin with information from the medical interview and the results of the blood smear examination and a Coombs' test. From these data, five groups of patients can be distinguished.

1. *Those in whom the diagnosis is clear because of obvious exposure to infectious, chemical, or physical agents (page 740).*

2. *Those with Coombs-positive hemolytic anemia.* Such individuals may be presumed to have immunohemolytic anemia (Chapter 27). The subsequent investigation requires a search for an underlying disease as well as a serologic study of the nature of the antibody.
3. *Those with Coombs-negative, spherocytic hemolytic anemia.* Such patients probably have hereditary spherocytosis (Chapter 21). It is appropriate to confirm the presence of spherocytes by means of the osmotic fragility test and also to attempt to establish the familial nature of the illness by studying family members. Some unusual causes of spherocytosis may need to be considered if family studies provide no insight; immunohemolytic anemia may be associated with spherocytosis and is occasionally associated with a negative Coombs' reaction (page 913). Exposure to chemical or infectious agents producing spherocytosis may not always be easy to establish.

*Those with other specific morphologic abnormalities of erythrocytes.* The significance of various types of poikilocytes was discussed in a previous section (page 733) and does not need to be recapitulated in detail. Some poikilocytes, eg, elliptocytes and sickle cells, are virtually pathognomonic findings. Others, such as extensive red cell fragmentation, identify a category to which several diseases belong, as discussed in Chapter 28.

*Those with no specific morphologic abnormalities and a negative reaction to Coombs' test.* With these patients, a battery of screening tests should be performed, including hemoglobin electrophoresis (page 805), the heat denaturation test for unstable hemoglobin disease (page 808), the ascorbate-cyanide test (page 736), spot tests for deficiencies of pyruvate kinase (page 773) and glucose-6-phosphate dehydrogenase (page 788), and a screening test for paroxysmal nocturnal hemoglobinuria (page 961).

all of these procedures yield normal results,

making the diagnosis is likely to be difficult. One of the rarer erythrocyte enzyme deficiencies (Chapter 22) is possible, but these can be established only by specific assay.

## Therapy

Precise management of hemolytic anemia depends on the diagnosis. Thus, an attempt must be made immediately to ascertain the cause of the hemolytic anemia and to treat it if possible. Further exposure to any possible chemical, drug, or other etiologic agent must be stopped, or, if a bacterial agent is the cause, the infection should be treated specifically. Appropriate treatment of patients with "symptomatic" hemolytic anemias will need to be considered according to the nature of the underlying disease. Other more or less specific modes of therapy will be considered in sections dealing with specific entities. There are, however, certain generalizations that apply to a large proportion of the hemolytic diseases. These will be considered here.

A patient having an acute attack of hemolysis is treated by the use of appropriate measures to relieve shock, if present, maintain fluid balance, and allow renal repair, if the kidneys have been damaged, as fully outlined elsewhere. Blood transfusions, so useful in acute anemia of other types, must be employed with caution in hemolytic anemias, for, even when great care is taken in matching the blood, destruction of the transfused blood with an increase in the burden on the excretory organs and sometimes with ensuing thromboses may occur. Nevertheless, when blood destruction is rapid, the dangerous consequences of shock can only be met by administration of adequate amounts of blood. The presence of antibodies makes matching of blood difficult. Whenever possible, the patient's blood should be genotyped before the first transfusion and the specificity of his antibodies determined. Blood homologous as to ABO and Rh blood groups should be matched with that of the patient and the best match used. It should be ascertained that the recipient's serum does not contain hemolysins against a prospective donor's

bating them in a test tube at 37° C for one hour. In spite of all such precautions, transfusion may nevertheless accelerate hemolysis. In such cases, exchange transfusion may be required.

Since the spleen often is a major site of red cell destruction in hemolytic disorders, *splenectomy* may bring about relief or improvement in these illnesses. There is considerable variation in the response to splenectomy in the various hemolytic disorders. As a general rule, mildly damaged red cells are removed predominantly by the spleen, whereas more severely injured cells are destroyed by many parts of the RES, especially the liver (page 204). Therefore, patients with diseases associated with mild red cell defects are the ones most likely to respond to splenectomy.

The best and most consistent response to splenectomy occurs in persons with hereditary spherocytosis. Following this operation, all signs of hemolysis disappear although spherocytosis is unaltered (Chapter 21). A similarly excellent response has been observed in patients having hereditary elliptocytosis with an associated hemolytic process (page 761). Anemia associated with certain of the red cells' enzymatic deficiencies may lessen following splenectomy, but the response is only partial, and continuing signs of hemolysis are to be expected, such responses have been observed in patients with deficiencies of pyruvate kinase, hexokinase, or glucose-phosphate isomerase (Chapter 22). In unstable hemoglobin disease (Chapter 24), patients with mild to moderate disease improve after splenectomy, but those with severe disease do not.

In persons with immunohemolytic anemia, splenectomy may be effective not only because the spleen is a site of erythrocyte destruction but also because it may be a source of antibody. In general, patients with warm antibodies are more likely to respond than those with cold antibodies, patients with splenomegaly respond better than those without a palpable spleen, and those with incomplete antibodies, especially if present in relatively small amounts, are more likely to respond favorably to splenectomy than those

with complete agglutinins or complement-fixing antibodies. Although these generalizations are helpful, it nevertheless remains true that the results of splenectomy are quite unpredictable. This has led to efforts to develop methods for assessing the erythroclastic function of the spleen in the individual patient. Such methods are based on the injection of <sup>51</sup>Cr-labeled erythrocytes and monitoring the radioactivity over the spleen and liver with a unidirectional scintillation counter. Excessive splenic sequestration suggests that the spleen is playing a major role in hemolysis and that its removal may be beneficial (Chapter 27).

*Steroid hormones* are of great value in therapy of patients with certain forms of immunohemolytic anemia. Principles regarding their use are discussed in Chapter 27.

In patients with chronic hemolytic disorders, *folic acid* may sometimes be useful to prevent megaloblastic crisis (Chapter 14). A dose of 0.15 to 0.3 mg per day is ample for this purpose. Such therapy is particularly indicated in patients with severe, continuing hemolysis, in those subsisting on marginal diets, and in those who become pregnant.

## Acquired Hemolytic Anemia Resulting from Infections, from Chemical Agents, and from Physical Agents

The great majority of cases of acquired hemolytic anemia result from either antibodies directed against the red cell (Chapter 27), fragmentation of the erythrocyte (Chapter 28), or paroxysmal nocturnal hemoglobinemia (Chapter 29). There remains a group of hemolytic disorders, most of which are relatively uncommon, that are associated with infectious, chemical, or physical agents that damage the red cell directly. These disorders will be discussed in this section.

### Infectious Agents

Infectious agents may induce hemolytic anemia in several ways: (1) they may precipitate a crisis in a preexisting, compensated



hemolytic state (page 723); (2) certain viral and mycoplasma microorganisms appear to bring about the formation of hemolytic auto-antibodies (Chapter 27); (3) infection may precipitate hemolysis in patients with glucose-6-phosphate dehydrogenase deficiency (Chapter 23); and (4) the infectious agent may damage the erythrocyte directly. Only the last category will be considered here, as well as some instances in which pathogenesis is obscure.

## Malaria

Malaria is an acute, chronic, or recurrent febrile disease caused in man by four species of *Plasmodia*: *P. vivax*, *P. falciparum*, *P. malariae*, and *P. ovale*. Another species, *P. knowlesi* causes malaria in rhesus monkeys. These protozoan microorganisms are capable of parasitizing erythrocytes and certain other body tissues. Malaria is spread by mosquitoes of the genus *Anopheles*, and its semitropical and tropical endemic distribution corresponds to the distribution of this vector. Endemic areas no longer exist in the USA, although sporadic cases and small epidemics have been associated with Armed Forces personnel returning from endemic areas. The major clinical manifestations of the illness vary with the species and strain of *Plasmodium*, but the most prominent features are recurrent paroxysms of chills and fever associated with malaise, headache, and other systemic symptoms. Jaundice and hepatosplenomegaly may develop late in the course of the illness.

Mild anemia is a common sign of malaria. More severe anemia (VPRC less than 0.35 l/l) is observed in approximately 20% of patients with *falciparum* malaria.<sup>150</sup> The anemia appears to be a direct consequence of red cell invasion by the parasite. Erythrocyte life span is shortened considerably,<sup>150</sup> osmotic fragility is increased,<sup>177</sup> and haptoglobin disappears from the serum.<sup>147</sup> The hemolysis is accounted for in part by splenic removal and destruction of parasitized cells.<sup>176</sup> In addition, "pitting" of the parasite from the erythrocyte may occur, the cell being damaged in the process and consequently becoming more

susceptible to subsequent destruction. A lesion of nonparasitized erythrocytes, detected by means of scanning electron microscopy, was interpreted to be a cellular "scar" formed during the "pitting" process.<sup>142</sup> Such a lesion might explain the increased osmotic fragility and increased susceptibility to destruction of nonparasitized cells.

Anemia in malaria may also result in part from relative marrow failure (page 717), for the percentage of reticulocytes, which tends to be low during active infection, increases transiently following effective treatment. In *vivax* malaria, however, this phenomenon may be explained in part by an increased affinity of the organism for reticulocytes.<sup>182</sup>

The most serious hematologic complication of malaria is acute intravascular hemolytic anemia (*blackwater fever*), which occurs as a rare event in the course of infection by *P. falciparum*. The clinical manifestations are fulminating, the intravascular hemolysis being associated with prostration, vomiting, chills, and pyrexia. Hemoglobinemia, methemalbuminemia, hemoglobinuria, and hyperbilirubinemia are consistent features. In the most severe episodes, acute oliguric renal failure supervenes.

The pathogenesis of blackwater fever remains uncertain, and the present-day rarity of the disease has made adequate study by modern techniques difficult. Blackwater fever does not appear to reflect an unusual degree of parasitemia; in fact, characteristic ring forms often are absent from erythrocytes during the attack.<sup>156</sup> Europeans who have had repeated attacks of malaria and who have taken quinine irregularly have been the chief victims of the complication.<sup>146,156</sup> Often the disease appears to have been precipitated by taking quinine (Fig. 20-5), perhaps suggesting an immunologic reaction of the "innocent bystander" variety (page 917). Evidence supporting an autoimmune explanation for blackwater fever is largely indirect<sup>183</sup>; most attempts to demonstrate red cell antibodies have failed, but a few instances associated with positive reactions to Coombs' tests have been reported.<sup>141</sup> Some episodes thought to represent blackwater fever may have resulted from the use of primaquine-like drugs



*verruca peruana*, a benign condition characterized by wart-like lesions on the skin.<sup>175</sup> With Oroya fever, it is classified as *Carrión's disease*, named after the student who lost his life while investigating it.<sup>175</sup> Patients with *Bartonella* infection can be treated effectively with penicillin and other antibiotics.

*Bartonella muris* is found in rats (Fig. 45-1, page 1409). Anemia due to related organisms has been described in the dog and other animals as well as in rats. An interesting feature of the infection in animals is that anemia rarely occurs in the normal animal; instead, it develops in splenectomized rats<sup>171</sup> or in dogs receiving a deficient diet or deprived of plasma substances by plasmapheresis.<sup>165, 171</sup> It has been clearly demonstrated in the animal studies that the anemia is due to blood destruction.<sup>182</sup>

### *Clostridial Sepsis*

*Clostridium welchii* (*Cl. perfringens*) septicemia, usually following septic abortion,<sup>52, 166</sup> but sometimes occurring in association with a diseased biliary tree,<sup>144</sup> or leukemia,<sup>148</sup> quite regularly produces profound hemolytic anemia. Signs of intravascular hemolysis are prominent, and many microspherocytes are found in the blood. The hemolysis probably results from the elaboration of clostridial  $\alpha$ -toxin,<sup>161</sup> a lecithinase that acts upon the erythrocyte membrane lipids to form highly lytic lysolecithins.

### *Other Bacterial Infections*

Only a few instances of hemolytic anemia have been encountered in most other bacterial infections. Hemolysis has been reported in children suffering from streptococcal, staphylococcal, or pneumococcal septicemia or endocarditis.<sup>41, 149, 152, 157, 169</sup> Hemolytic anemia could be induced by intradermal injections of living pneumococci in rabbits.<sup>178</sup> Hemolytic anemia and thrombocytopenia were noted in a patient with meningococcemia.<sup>170</sup>

Certain gram-negative bacillary infections have occasionally been associated with

hemolytic anemia. Intravascular hemolysis with hemoglobinuria has been observed in patients with *cholera*.<sup>155</sup> Hemoglobinuria has been reported as occurring in patients with typhoid fever,<sup>172</sup> and a milder hemolytic state is a well-known, though uncommon, complication of this illness.<sup>145, 161, 172</sup> Hemolysis has only rarely been reported with other *Salmonella*<sup>153, 154</sup> infections or with *E. coli* infections.<sup>160</sup> The hemolytic anemia noted in patients with *H. influenzae* meningitis was ascribed to anti-erythrocyte antibodies in the rabbit antisera used in treatment, rather than to the direct effects of the microorganism.<sup>167</sup>

Severe hemolytic anemia has been observed occasionally in patients with *tuberculosis*, but the pathogenesis is obscure. In several of such patients, the tuberculosis was in the generalized, miliary stage<sup>41, 163</sup> or involved the spleen,<sup>158, 159</sup> but in others it was confined to the chest.<sup>166, 179</sup>

Hemolytic anemia has been noted in a patient with relapsing fever, caused by the spirochete *Borrelia recurrentis*.<sup>143</sup>

## **Chemical Agents, Drugs, and Venoms**

### *Oxidant Drugs and Chemicals*

A number of chemical agents can bring about the oxidative denaturation of hemoglobin, leading to the sequential formation of methemoglobin, sulfhemoglobin, and Heinz bodies (page 204). In some cases, the chemical itself acts as an oxidizing agent, but, more frequently, it interacts with oxygen to form free radicals or peroxides. These, if produced in quantities too great to be detoxified by the glutathione-dependent reduction system (page 103), are the substances that damage hemoglobin and other cellular structures.

Individuals deficient in glucose-6-phosphate dehydrogenase (G6PD) or other components of glutathione-dependent detoxification processes (Chapter 23) are particularly sensitive to the hemolytic effects of "oxidant" compounds (Table 23-2, page 785). It is also true, however, that some of these agents are powerful enough to overcome the defense mechanisms of apparently normal erythro-

cytes. Others produce hemolysis if given to normal subjects in higher than usual doses or if renal failure leads to unusually high blood levels

From reports submitted to the AMA registry on adverse drug reactions, the most common drugs and chemicals implicated in hemolysis in normal subjects appear to be naphthalene (mothballs), nitrofurantoin (Furadantin), salicylazosulfidine (Azulfidine), sulfamethoxypyridine (Kinex), aminosalicilic acid, and sodium sulfoxone.<sup>202</sup> Data from other sources give evidence of the relatively high frequency of reactions to various sulfonamide compounds.<sup>221-231</sup> Other agents reported to cause "oxidative" hemolysis in apparently normal subjects include phenacetin,<sup>219</sup> sulfones,<sup>219-223</sup> phenylsemicarbazide,<sup>231</sup> resorcin,<sup>217</sup> phenylhydrazine and its derivatives,<sup>201-203</sup> aniline,<sup>232</sup> hydroxylamine,<sup>231</sup> nitrobenzene,<sup>233</sup> phenol derivatives,<sup>216</sup> and potassium or sodium chlorates.<sup>211-224</sup>

Molecular oxygen may cause hemolysis under certain circumstances.<sup>230</sup> A mild hemolytic reaction was observed in an individual treated for stroke with oxygen at high pressure.<sup>235</sup> Over a six-day period following the exposure, the VPRC fell from 0.48 to

0.35 l/l, the reticulocytes increased from 0.5 to 4.6%, and the bilirubin rose to 1.6 mg/dl. Hemolysis also has been induced in mice by exposure to oxygen, and the intensity of the reaction was greatly exaggerated in vitamin E-deficient animals (Chapter 4). In normal human subjects only four hours of exposure to 100% oxygen induced an increase in plasma hemoglobin levels to about 25 mg/dl, and abnormalities in erythrocyte osmotic fragility were detected.<sup>230</sup> It is likely that the toxic effects of oxygen are related to the generation of  $H_2O_2$ ,<sup>235</sup> and the peroxidation of membrane lipids.<sup>214</sup>

### Chemicals Producing Hemolysis by Nonoxidative Mechanisms

*Arsine* (arsenitretted hydrogen) is the name given to a highly toxic mixture of gases incorporating arsenic and hydrogen, of which the most common is  $AsH_3$ . Arsine has certain industrial uses, eg, in the manufacture of bleaches, of fertilizers,<sup>219</sup> and in tin-mining. It also evolves whenever nascent hydrogen is generated in the presence of arsenic, as can occur unsuspectingly when acids contact certain ores and waste materials containing metals and traces of arsenic.<sup>226</sup> The impor-

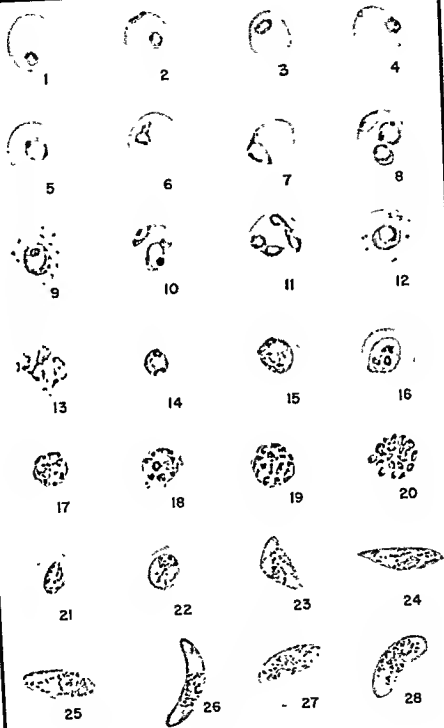
## PLATE XI

### *P. falciparum*

1. Very young ring form trophozoite
2. Double infection of single cell with young trophozoites, one a "marginal form," the other a "signet ring" form
- 3 4 Young trophozoites showing double chromatin dots
- 5 6 7 Developing trophozoite forms
8. Three medium trophozoites in one cell
- 9 Trophozoite showing pigment, in a cell containing Maurer's spots
- 10 11. Two trophozoites in each of two cells, showing variation of forms which parasites may assume
- 12 Almost mature trophozoite showing haze of pigment throughout cytoplasm. Maurer's spots in the cell
- 13 Aestivo-autumnal slender forms
14. Mature trophozoite, showing clumped pigment
15. Parasite in the process of initial chromatin division
16. 17 18. 19 Various phases of the development of the schizont ('presegmenting schizonts')
20. Mature schizont
21. 22. 23. 24 Successive forms in the development of the gametocyte—usually not found in the peripheral circulation
- 25 Immature macrogametocyte
26. Mature macrogametocyte
27. Immature microgametocyte
28. Mature microgametocyte

(Reproduced with permission from the *Manual for the Microscopical Diagnosis of Malaria in Man*, National Institutes of Health Bulletin No 180 [by Anne Wilcox])

# PLATE XI



INEZ DEMONET

tant acute toxic effects of arsine include renal and hepatic damage and acute hemolytic anemia, which begins within a few hours of exposure. Signs of intravascular hemolysis are prominent, and severe anemia may develop in the absence of morphologic changes in the red cells. Leukocytosis is a usual feature. A mortality rate of 22.5% was recorded in the past.<sup>204</sup> A more chronic form of arsine poisoning occurring in gold-refiners was associated with severe hemolytic anemia, prominent stippling, erythroblastosis, and rapid recovery when exposure was interrupted.<sup>205</sup> The way in which arsine damages erythrocytes is poorly understood.<sup>226</sup>

Hemolytic anemia can occur as a result of the direct effects of *inorganic copper* on the red cell. Such episodes have been noted rarely in persons<sup>207,215</sup> and animals<sup>218,245</sup> exposed to toxic amounts of copper sulfate. Copper also has been implicated in hemolytic episodes following hemodialysis.<sup>228,233</sup> In addition, hemolytic anemia occurring during the course of Wilson's disease results from the release of large amounts of inorganic copper into the blood stream.<sup>213</sup> A number of abnormalities can be induced by exposure of erythrocytes to copper salts *in vitro*,<sup>213,236</sup> including increased autohemolysis, thermolability of hemoglobin, decreased glutathione levels, Heinz-body formation, and decreased activity of various enzymes associated with the hexose monophosphate shunt. The relation of these abnormalities to hemolysis *in vivo* is unclear, however.

Hemoglobinuria and even death from renal failure have been observed in association with transurethral resection of the prostate, apparently as the result of penetration of the irrigating fluid, *distilled water*, into the blood stream via lymphatic and venous channels opened by the operation.<sup>229</sup> The entry of more than 0.6 liter of water into the circulation will produce hemoglobinemia and hemoglobinuria as a result of osmotic hemolysis. This is probably the cause of the hemolysis noted in survivors of near drowning in fresh water.<sup>241</sup> In such situations the normal protective mechanisms (page 205) are overwhelmed.

Hemolytic anemia resulting from drug-induced immune reactions is discussed in Chapter 27 (page 916).

### Venoms

Fewer than 20 cases of hemolytic anemia have been reported in association with *spider bites*. In most instances, the spider has not been specifically identified, but clinical and epidemiologic data strongly implicate the brown recluse spiders, *Loxosceles reclusus* and *Loxosceles laeta*,<sup>239</sup> which inhabit South America and the Central and Southern United States. Their initially painless bite develops into a painful, edematous, necrotic lesion that may progress to an extensive, slowly healing, gangrenous process (necrotic arachnidism). In a minority of patients, typical, acute, intravascular, hemolytic anemia develops within several hours to five days after being bitten. Hemoglobinuria and severe anemia are characteristic findings; spherocytes, anisopoikilocytosis, stippling, and leukocytosis are found in the blood; and mechanical and osmotic fragility of the erythrocytes is increased. The hemolytic episode subsides spontaneously in about one week. The nature of the hypersensitivity of the patients developing the hemolytic reaction has not been determined; in at least one patient the possibility of glucose-6-phosphate dehydrogenase deficiency was excluded.<sup>239</sup>

*Snake venoms*, especially cobra venom, hemolyze red cells *in vitro*, the hemolytic activity depending upon two constituents, a phospholipase and a basic protein called "direct lytic factor."<sup>206</sup> Hemolytic anemia is a relatively uncommon manifestation of cobra bites, possibly because critical amounts of venom are not often injected into the blood stream. Of 47 patients bitten by a cobra, only 6 developed systemic toxicity. Three of these developed a mild to moderate hemolytic anemia characterized by a fall in blood hemoglobin concentration of 3 to 4 g/dl, reticulocytosis, hyperbilirubinemia, and leukocytosis.<sup>242</sup> No signs of intravascular hemolysis were observed. On the other hand, red cell abnormalities were found in all of a group

of five patients who were bitten by vipers or cobras in India.<sup>240</sup> Spherocytes were prominent, comprising 30 to 50% of red cells by three to four days after the patients were bitten. The plasma hemoglobin ranged from 0.02 to 0.06 g/dl, and hemoglobinuria occurred in two of the patients. Other abnormalities included falsely positive reactions to Coombs' tests, acanthocytosis, the presence of Heinz bodies, and erythroblastosis. Blood hemoglobin levels decreased to as low as 10 g/dl.

Hemolytic reactions to *bee stings* appear to be very rare. In one instance, a three-year-old child who was stung 200 to 300 times by honey-bees died following the development of intravascular hemolysis and oliguric renal failure.<sup>203</sup>

## Physical Agents

### Thermal Injury

Acute hemolytic anemia has been observed following extensive thermal burns.<sup>237,243</sup> Signs of intravascular hemolysis are associated with schistocytes and spherocytes in the blood and increased osmotic and mechanical fragility of the erythrocytes.<sup>243,246</sup> The hemolysis occurs during the 24- to 48-hour period following the burn,<sup>246</sup> and the severity of the reaction is related to the area of body surface affected. Thus, hemoglobinuria was found in 11 of 14 moderately to severely burned patients, and in most of these more than 15% of the body surface was involved.<sup>243</sup> In another study, hemolysis was apparent in patients with third-degree burns affecting more than 20% of the body surface.<sup>225</sup> As much as 30% of the circulating red cell mass may be destroyed in a two-day period in severely burned individuals.<sup>210,247</sup> Following the acute hemolytic process, anemia develops and may last for many weeks.<sup>231</sup> After the first 48 hours, however, signs of hemolysis are not prominent, and it is likely that these later stages of the anemia of thermal injury are a form of the anemia of chronic disorders (Chapter 18).

The acute hemolytic reaction probably re-

sults from the direct effects of heat on erythrocytes. When red cells were heated *in vitro* to temperatures greater than 47° C, irreversible morphologic and functional abnormalities occurred, the severity of which was related to the temperature and the duration of exposure.<sup>220,227</sup> The major alterations were fragmentation of the cells and the development of spherocytes, accompanied by an increase in osmotic and mechanical fragility. Furthermore, when such cells were injected into experimental animals, prompt hemoglobinemia and hemoglobinuria were observed with selective removal of the abnormal cells within a few hours.<sup>220</sup> With <sup>51</sup>Cr, it could be demonstrated that mildly heat-damaged erythrocytes are removed predominantly by the spleen, but, with greater degrees of damage, the isotope accumulates in the liver.<sup>227,248</sup>

### Ionizing Irradiation

Very high doses of total body irradiation (180 to 600 r) resulted in reduced erythrocyte survival in dogs.<sup>241</sup> This effect did not seem to result from direct radiation-induced damage to red cells, since reduced survival of even donor cells occurred in previously irradiated animals. In *in vitro* studies, red cells were found to be very resistant to radiation damage, doses in the range of 20,000 r being required to produce detectable effects.<sup>212</sup> However, *in vivo* radiation in mice resulted in a tendency of erythrocytes to agglutinate spontaneously in saline solution and to undergo hemolysis in acidified serum.<sup>222</sup> Despite these observations, no clear evidence that irradiation induces hemolytic anemia in man has been presented.

## Acquired Hemolytic Anemia Associated with Hypophosphatemia

Severe spherocytic hemolytic anemia (VPRC 0.25 l/l, reticulocytes 8%, t<sub>1/2</sub> Cr 4.5 days) was detected in a patient with profound hypophosphatemia<sup>15</sup> (serum phosphorus, less

than 0.1 mg/dl). Increased rigidity of the erythrocytes was associated with an 11 to 30% reduction in important intracellular phosphorylated compounds, principally ATP and 2,3 DPG. All of the abnormalities returned to normal after parental phosphate administration.

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## *Hereditary Spherocytosis and Other Hemolytic Anemias Associated with Abnormalities of the Red Cell Membrane*

Hereditary Spherocytosis  
 Hereditary Elliptocytosis  
 Acanthocytosis  
 Abetalipoproteinemia  
 Other Disorders Involving the Red Cell Membrane  
 Stomatocytosis  
 Hereditary Nonspherocytic Hemolytic Anemia with Altered Erythrocyte Phospholipid Composition  
 LCAT Deficiency  
 "Infantile Pyknocytosis"

### Hereditary Spherocytosis (HS)

#### Definition and History<sup>35,97</sup>

Hereditary spherocytosis is characterized by a variable degree of anemia, splenomegaly, spherocytosis, and increased osmotic fragility of the red corpuscles, as well as by jaundice, often of mild degree and unaccompanied by bile in the urine (acholuric). HS is inherited as an autosomal dominant trait, but the clinical manifestations may be so mild that the disease may sometimes not be recognized even until late adult life and the true extent of familial involvement may be appreciated for the first time only after thorough examination of family members has been carried out, rather than from their history alone. Earlier commonly used synonyms, *congenital hemolytic jaundice* and *chronic familial jaundice*, emphasize the prominence of the jaundice in many HS patients.

The earliest observations concerned with hemolytic anemias, including HS, were described in Chapter 20 (page 718). The studies

THE pathogenesis and classification of hemolytic anemias of various kinds were discussed in Chapter 20. Here those conditions in which the cause of the increased red cell destruction has been found to reside in the red cell membrane will be considered. Of these, hereditary spherocytosis is the prime example, but several less common conditions will also be discussed. Noteworthy is the fact that all of these disorders are characterized by observable morphologic abnormalities of the red cell.

of Haden<sup>18</sup> and of Castle and Daland<sup>14</sup> subsequently drew attention to the possibility that a structural abnormality is the underlying defect in HS. The relation of the spleen to the manifestations of this disease and the reasons for the curative role of splenectomy were clarified by the studies of Young and his associates<sup>112</sup> and of other investigators, as will be described below (page 757).

### Prevalence

HS probably is the most common of the hereditary hemolytic anemias among people of Northern European descent. In the United States the incidence is approximately 220 per million.<sup>79</sup> On the basis of the study of family trees extending through three or four generations<sup>31,43,111</sup> it is clear that the HS gene is transmissible through either parent<sup>71,87</sup> but penetrance may be incomplete, as is ascertainment.<sup>79</sup> Consequently, somewhat fewer than half the siblings have been found to be affected (0.45).<sup>72,79</sup> Homozygosity for the trait, never certainly established, was presumed in a family in which all 13 children were affected; 9 of these children manifested physical or mental retardation.<sup>8</sup> Sporadic cases, with both parents unaffected, are rare.<sup>31,87,111</sup>

HS is uncommon in Negroes,<sup>66</sup> but has been found even in African Bantu.<sup>75</sup> Males and females are affected equally. The condition has been recognized at all ages, in the form of severe jaundice at birth,<sup>71,91,109</sup> and even in old age (77 years).<sup>87</sup> The extremes are unusual, however. In a series of 28 cases,<sup>111</sup> anemia or jaundice was first detected at age 10 or younger in 8 patients and between the ages of 10 and 45 years in 15, the age range of the remaining 5 patients having been from 47 to 54 years.

### Symptomatology

The symptoms are those characteristic of chronic congenital hemolytic anemia (Chapter 20, page 722), but they vary greatly, both in regard to their time of onset and their severity. They are most often first noticed in

childhood or adolescence; in these young subjects the disease is likely to be more severe than in persons in whom it is first detected later in life. How much this disease may vary in severity is indicated by a study of 68 out of 161 members of a family comprising three generations; 11 of the 68 showed all the manifestations of the disease, 34 had the disease in a well-compensated form without anemia, in 13 the manifestations were mild, and 10 were completely healthy.<sup>43</sup> Because the signs of HS may be subtle, a study based only on family history, that does not include examination of the relatives of a patient, cannot be regarded as being negative as far as inheritance of the disorder is concerned.

Icterus usually is not intense, and is said to increase with fatigue, cold, emotion, or pregnancy. There may be no detectable jaundice despite other signs of active hemolysis,<sup>43,71,72,97</sup> or there is a constant sallow complexion, but even when icterus is well marked the patients are generally "more yellow than sick." The jaundice, no matter how intense, is not accompanied by itching of the skin, bradycardia, or xanthoma. Once severe jaundice and anemia have developed, complete spontaneous remission is unusual.<sup>96</sup>

From time to time the jaundice may deepen and the anemia increase. Abdominal pain, vomiting, tachycardia, and fever may be associated.<sup>31</sup> In addition, at any time, even for the first time in late adult life, there may be a sudden, acutely developing illness, the "*crise de déglobulization*" (Chapter 20, page 723). Such episodes<sup>31,90</sup> had been attributed to a sudden acceleration of blood destruction, but Owren,<sup>82</sup> studying four cases occurring in members of the same family, was impressed by the disappearance of reticulocytes from the blood, and the presence of leukopenia, thrombocytopenia, and acute aplasia of the erythropoietic tissue in the bone marrow (Fig. 20-1). As discussed in Chapter 20, such *acute erythroblastopenia*<sup>41</sup> or aplasia may develop at the same time in several affected members of a family.<sup>28,47,73</sup> The crisis may last from 5 to 12 days.

Symptoms of *biliary tract disease* may first bring the patient to the physician. Cholelithia-

sis has been found in 43<sup>4</sup> to 85%<sup>111</sup> of the patients and has been described as present even in patients as young as 3 years of age.<sup>42</sup> An uncommon complication, which may nevertheless be the chief complaint, is chronic leg ulcer<sup>38,95</sup> (Chapter 20, page 723). Sometimes chronic dermatosis<sup>13</sup> and pigmentation (Fig. 21-1) may be the only indications of a healed chronic ulcer.

The *spleen* is almost always enlarged when the disease is active and may be huge. In extensive family studies, the spleen was found to be palpable in 75<sup>71</sup> to 82%<sup>111</sup> of affected individuals. Its size has not been found to be related to the severity of the disease.<sup>71</sup>

The liver may not be palpable or it may extend 1 to 3 cm below the costal margin. The finding of an exceptionally large liver should arouse suspicion of a complication or an error in diagnosis.

Nosebleeds are common in childhood, but hemorrhage from other parts of the body, as in Banti's syndrome or in cirrhosis of the liver, does not occur. Sometimes the lymph nodes may be enlarged.<sup>31</sup> Neural symptoms have been reported,<sup>24</sup> but they must be exceedingly rare.

The tower skull ("Turmschädel") is the most common skeletal abnormality that may be associated with HS,<sup>111</sup> and thickening and striation of the frontal and parietal bones may be noted on x-ray examination.<sup>13</sup> Changes in the bones that are similar to those present in sickle cell anemia and thalassemia, though less marked, may be seen. X ray may reveal masses of heterotopic bone marrow (page

757) alongside the vertebral column, and these may be mistaken for tumors.<sup>21,50,54</sup>

More than two dozen different congenital anomalies have been described in patients with HS, including prominent eyes,<sup>31</sup> epicanthus, persistent pupillary membrane, abnormally wide root of the nose, persistence of deciduous teeth in adults, misplacement of permanent teeth, palatal deformities, polydactylia, and brachydactylia.<sup>43,51</sup> It is not certain, however, that the frequency of these anomalies exceeds that in the population at large.

If symptoms commence in childhood, growth may be impaired.<sup>31,43</sup> Infantilism may occur<sup>8</sup> and other endocrine disorders have been observed.<sup>41</sup>

## Laboratory Manifestations

### The Blood

Hemoglobin levels between 9 and 12 g/dl are most common, but anemia may not be present, or it may be quite severe. A rapid fall to 3 or 4 g/dl may occur during a crisis, and, even in the absence of anemia, hemoglobin values as low as 6 g/dl may be observed and may be maintained for a long time. The reduction in red cell count, hemoglobin level, and volume of packed red cells may or may not be proportional. MCV may be normal, increased, or greatly decreased. In a series of 76 affected, nonsplenectomized individuals the MCV was found to be  $83 \pm 8.5$  fl.<sup>72</sup> Values between 77 and 87 fl are common, but the MCV may be as low as 62 fl or as high as 125 fl. Macrocytic anemia is more likely to occur in subjects with very severe anemia and pronounced reticulocytosis. Variations in MCH usually correspond to changes in volume, but, characteristically, the MCHC is high (37 to 39 g/dl) (page 119).

Variation in the size of the red corpuscles and the presence of macrocytes have even led to confusion of HS with pernicious anemia in some instances. The mean diameter of the red cells is reduced, owing to the presence of round, fully hemoglobinized cells of small



Fig 21-1. Marked pigmentation of the skin about the ankles in a patient with hereditary spherocytosis

size (Fig. 21-2). These microspherocytes, even when as small as 4  $\mu\text{m}$  in diameter, show no central pallor because of their spherical rather than biconcave shape. Their diameter in stained smears averages 6.2 to 7.0  $\mu\text{m}$  (range 4.0 to 7.6  $\mu\text{m}$ ). In wet films of blood it also is below normal.

The unusual thickness<sup>18,96</sup> of the red corpuscles, 2.2 to 3.4  $\mu\text{m}$  instead of 2.0  $\mu\text{m}$  or less as in normal blood<sup>103</sup> (Fig. 21-3), accounts for the low mean cell diameter despite normal or only slightly reduced MCV. The degree of spherocytosis varies from patient to patient.

*Reticulocytes* are characteristically increased in number. Values between 5 and 20% are common, but they may be as high as 50 and even 92%,<sup>5</sup> or as low as 2%. In a series of 76 affected nonsplenectomized members of a family of 180, the mean reticulocyte count was  $9.9 \pm 4.9\%$ .<sup>71</sup> Polychromatophilia often will be observed in variable degree, as well as occasional normoblasts and micronormoblasts. During recovery following a crisis, such signs of active erythropoiesis are particularly prominent. Poikilocytosis is rarely very marked, although structures suggestive of fragmented red corpuscles may be seen.

The *fragility* of the red corpuscles in hypotonic saline solutions is increased in typical

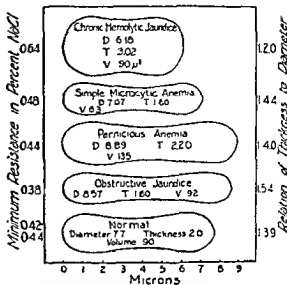


Fig 21-3. Diagram representing cross section of erythrocytes in different clinical conditions. D refers to mean diameter, T means thickness and V means corpuscular volume (From Haden,<sup>49</sup> courtesy of the author and American Journal of Medical Sciences)

cases. The saline concentration at which hemolysis begins ranges from about 0.51 to 0.72 g/dl and may be as high as 0.87 g/dl. Hemolysis may be complete at the concentration where it normally commences. If the amount of hemolysis in various strengths of

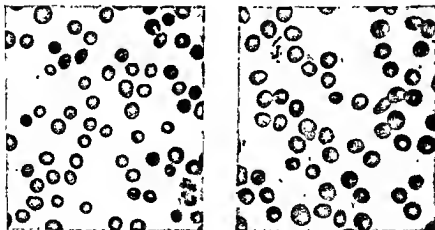


Fig. 21-2 Blood smears from two patients with hereditary spherocytosis. There were many more spherocytes (the small, round, dark cells) in the smear on the left than in that on the right. Note the lack of poikilocytosis (Wright's stain,  $\times 800$ )

saline solution is plotted as a curve (Fig. 20-4, page 734), it may be found that the curve of hemolysis is normal in shape but shifted to the left; it may be "tailed" with only a small proportion of the red corpuscles hemolysing in saline concentrations greater than 0.45 g/dl<sup>25</sup>; or increased osmotic fragility may not be demonstrable until after 24-hour incubation of the cells.<sup>110</sup> There is no correlation between the degree of anemia and the fragility of the corpuscles, but osmotic fragility, measured under controlled conditions, was shown to be a precise measure of the degree of spherocytosis at the time of exposure to the hypotonic medium.<sup>14,37</sup> Osmotic fragility may be only minimally abnormal during an aplastic crisis.<sup>56</sup>

Mechanical fragility is greater than normal.<sup>111</sup> With rare exceptions,<sup>57,68,113</sup> the spontaneous autohemolysis, at 37° C, of sterile blood of HS patients occurs sooner and in greater degree than does the blood from normal persons<sup>92</sup> (10 to 50% instead of the normal 4%) or blood from patients with autoimmune hemolytic disease, except at times of very active blood destruction.<sup>111</sup> The addition of glucose or of ATP markedly reduces the degree of autohemolysis.<sup>92</sup>

Reaction to the Coombs' test usually is negative.<sup>111</sup> Reported positive reactions, when not attributable to the use of inadequately absorbed rabbit serum or inadequate characterization of the tested cases, may be explained, at least in some instances, by antibody development superimposed upon the original congenital disease.<sup>28</sup> If the reticulocyte count is high, a falsely positive reaction to Coombs' test may be due to the transferrin bound to the reticulocytes (page 160). Only minimal and irregular deviations in the activity of the enzymes of glycolysis are found.<sup>80</sup>

The number of leukocytes is usually normal or only slightly increased, but in an aplastic crisis it may be decreased and after a crisis there is marked leukocytosis and a "shift to the left." During the chronic stage of anemia, the numbers of lymphocytes, plasma cells, and basophils may be increased.<sup>43</sup> The platelet count usually is within

the normal range, although it may be somewhat increased or, more rarely, moderately reduced.

Serum bilirubin (indirect) is increased ( $1.6 \pm 1.1$  mg in one large series of cases<sup>72</sup>). (Fig. 20-3, page 728.) The icterus index may be as high as 100, but values of 15 to 30 are more common.

The plasma or serum iron value may be normal or increased.

### Urine

The urine may or may not contain increased amounts of urobilinogen, but bile pigments and bile salts are absent. Hemoglobinuria is very unusual. The stools contain excessive quantities of urobilinogen, as much as 5 to 20 times the normal.<sup>55,84</sup>

### Bone Marrow

In the bone marrow, erythropoietic hyperplasia of the normoblastic type is found. Normoblasts may comprise 25 to 60% of all the nucleated cells, and many mitotic forms are evident.<sup>69</sup> In the absence of complicating folate deficiency (page 579), megaloblasts of the type seen in pernicious anemia are not found, and the giant, abnormal leukocytes are also lacking.<sup>69</sup>

### Diagnosis

The presenting clinical picture may suggest a great variety of disorders that are characterized by jaundice,<sup>100</sup> fever, abdominal complaints, splenomegaly (Chapter 45), or anemia. Diagnosis depends on (1) establishing that hemolytic disease is present: anemia (usually), reticulocytosis, splenomegaly, unconjugated bilirubinemia and increased urobilinogen in the urine and stools; (2) detecting spherocytosis by examining the blood smear and measuring osmotic fragility; (3) excluding the possibility of other causes of spherocytosis; and (4) establishing the familial character of the condition.

The microspherocytosis is striking and the



cells are more uniform than those noted in immunohemolytic anemia or in other forms of hemolytic anemia that may be associated with spherocytosis, such as that following damage to the erythrocyte by chemical, infectious, or physical agents (Chapter 20). However, when the anemia is severe, macrocytosis may be marked. Nevertheless, there should be no confusion with pernicious anemia because HS patients usually are younger than pernicious anemia subjects and they do not have achlorhydria or neural involvement. Furthermore, in pernicious anemia, the reticulocytes are normal in number as a rule, except during the response to therapy. However, megaloblastic anemia may develop in HS subjects as in persons with other chronic hemolytic disorders, probably because of increased folate utilization associated with the net increase in the rate of DNA synthesis as the result of the marked and continuous erythroid hyperplasia (Chapter 14).<sup>63</sup> Such folate deficiency is more likely to occur when the diet has been deficient, when the patient has had repeated pregnancies, or when liver disease is present.<sup>67</sup>

Doubt may arise if the fragility of the red corpuscles is not found to be increased.<sup>25,43 110,114</sup> The value of incubation of the cells under these circumstances has been mentioned. Unless the diagnosis is clear-cut, the patient should be studied in the manner already described (page 737) in order to exclude the possibility of various other causes of hemolytic anemia (Table 20-3, page 721). The manifestations of acquired forms of hemolytic anemia usually are more severe than those of the hereditary forms. If there is a family history of anemia, jaundice, and/or splenomegaly, the various hereditary non-spherocytic hemolytic anemias, thalassemia, sickle cell anemia, and other hemoglobinopathies must be considered. It should be borne in mind that appearance of symptoms late in life does not exclude HS (page 752) and that a family history without appropriate examinations is not to be trusted. Constitutional hyperbilirubinemia (Table 20-6, page 729) has been confused with hereditary spherocytosis.

## Complications

The frequency of biliary tract disease has been mentioned previously. So also has the rare occurrence of chronic leg ulcers, megaloblastic anemia, and various developmental anomalies. Gout has been observed.<sup>97</sup>

The discovery of gallstones in a young person should cause one to suspect the existence of HS. If HS is diagnosed, splenectomy must be performed before or simultaneously with cholecystectomy, since the bilirubinemia requires correction if the deposition of bilirubin salts is to be avoided. Secondary hemachromatosis has been observed<sup>3</sup> and primary carcinoma of the gallbladder was encountered at the unusually early age of 36 years in a patient with HS.<sup>49</sup>

## Treatment

HS is the one disorder in which splenectomy is associated with almost uniformly beneficial and lasting results. Splenectomy is followed in a few days by fading of the jaundice and a gradual rise in hemoglobin and VPRC to normal levels, which are reached in several weeks.<sup>93</sup> The reticulocytes decrease and other signs of accelerated erythropoiesis disappear. Red cell survival increases<sup>2</sup> but does not become entirely normal.<sup>15</sup> Microcytosis often persists and spherocytes usually are still found in the blood smear.<sup>111</sup> The corpuscular fragility usually is unaltered.<sup>97</sup> However, in some patients, the blood findings are modified in the direction of normal.<sup>104</sup> The platelet count usually rises following splenectomy. Leukocytosis, even to  $48 \times 10^9/l$ , follows splenectomy immediately and persists in moderate degree for a long time<sup>29</sup> after operation.

Splenectomy should be advised in any patient who has typical HS if he has been continuously anemic or gives a history of hemolytic or aplastic crises. The operative mortality in good hands is extremely low. The presence of cholelithiasis would indicate the need for splenectomy. Chronic leg ulcers may clear up only after splenectomy.<sup>95</sup> It is difficult to see what is to be gained by delay-

ing operation unless the patient is completely compensated and has always been symptom free. Even in such subjects, one cannot be sure that this good fortune will persist and, in fact, development of biliary tract disease is to be expected. Only in infancy should the operation be delayed since the risk of infection is greater in the first year of life than later.<sup>11</sup> However, splenectomy in children with this disease does not carry the high risk that has been claimed (page 360) and operation at four to five years of age has the advantage that impairment of growth and risks from crises or other complications are avoided.

The use of anticoagulants postoperatively to prevent thrombosis is unnecessary, in spite of marked postoperative thrombocytosis.

Cases have been reported in which the dramatic improvement that usually follows splenectomy did not occur. In such cases the correctness of the diagnosis must be questioned. Failure to relieve the jaundice and anemia also raises the possibility that accessory spleens were overlooked; however, there are but three well-documented instances of such a finding on surgical exploration.<sup>70</sup>

Other measures are of no value in this condition. Iron is contraindicated, vitamin B<sub>12</sub> and corticosteroids are not helpful, and folic acid is useful only when there is complicating folate deficiency<sup>71</sup> (page 579). Blood transfusions are usually unnecessary except in a crisis.

### Prognosis

The illness generally is more serious when symptoms appear in childhood than in later life.<sup>33</sup> Death may occur during a crisis. Repeated attacks may be associated with impairment of bodily and sometimes mental development; cardiac decompensation may occur and biliary tract disease may ensue.<sup>33</sup> Many patients, however, have attained an advanced age in spite of chronic anemia.<sup>96</sup> The histories of some patients<sup>19</sup> suggest that there may be prolonged spontaneous improvement without recurrence of jaundice until late adult life, but, usually, once jaun-

dice has set in, it does not clear up completely until after splenectomy.

### Pathology

The spleen is enlarged, weighing 1000 to 1500 g, and is usually easily removed at operation. Adhesions are uncommon. The cut surface is relatively dry, dark purplish-red in color, homogeneous in texture, and bulges slightly above the capsule. The malpighian bodies cannot be distinguished. Microscopically they are found to be small and widely separated. The pulp is a mass of closely packed red cells which distend, distort, and dilate it. By light microscopy the sinuses appear empty, but electron microscopy shows them to contain red cells the majority of which have lost their hemoglobin.<sup>78</sup> There may or may not be increased iron pigment.<sup>100</sup> Cordal macrophages are increased and show active erythrophagocytosis, as do the sinus endothelial cells.<sup>78</sup> Unlike the finding in the spleen of Banti's syndrome, there is no thickening of the trabeculae.

The liver is not enlarged in most patients, nor is it usually cirrhotic, but the quantity of iron pigment may be increased in the hepatic and the Kupffer cells, and bilirubin stones may be found in the gallbladder. The kidneys often show well-marked hemosiderosis and the lymph glands may also contain pigment.

The bone marrow is strikingly hyperplastic, dark red in color, and free from fat. The microscopic picture is that of marked erythroid hyperplasia (page 755). Heteropia of the bone marrow may be found in the renal pelvis<sup>29</sup> or along the vertebral column.

### Pathologic Physiology and Pathogenesis

The fact that the manifestations of HS are the consequence of an inherited trait whereby red corpuscles of unusual thickness closely approaching spheres in shape are produced<sup>13</sup> is central to an understanding of this disease. HS cells have a configuration similar to that assumed by red corpuscles when they are about to burst in hypotonic solutions of so-

dium chloride (Fig. 21-4).<sup>45</sup> The vulnerability of these cells to destruction, especially in the spleen, results in the manifestations of hemolytic disease and, because this destruction is extravascular, such manifestations as hemoglobinemia and hemoglobinuria almost never occur. Because the normal bone marrow in case of need is capable of increasing red cell production six- to eight-fold (Chapter 20), anemia may be minimal or even entirely absent; only under special circumstances, not well understood, does compensation for the increased rate of destruction become impaired and "aplastic" crises develop.

The inherent defectiveness of the HS erythrocyte is shown by the fact that the survival of HS cells is greatly reduced even when the cells are transfused to normal individuals; on the other hand, normal cells survive normally in the circulation of persons who have inherited HS.<sup>26</sup> Yet the shape of

the cells is not of itself responsible for their destruction. Thus, when two nonanemic patients with HS were phlebotomized until their cells became hypochromic and thin, the cells' life span in the circulation was not improved.<sup>22</sup>

As described in Chapter 3, the red corpuscle possesses an energy-dependent system of active cation transport that requires a continuous supply of glucose. Incubation of normal cells in the absence of glucose leads eventually to depletion of ATP and loss of the mechanisms whereby membrane impermeability to cations is maintained. This need for glucose makes red corpuscles vulnerable to sequestration.

In the HS cell, glycolysis is not defective, as was once assumed.<sup>65-66</sup> However, for two reasons the HS cell is especially vulnerable to the effects of sequestration; these are an increased rate of  $\text{Na}^+$  flux<sup>9,32,39,108</sup> and depletion of membrane lipids. The heightened influx of  $\text{Na}^+$  stimulates an usually latent ATPase system.<sup>77</sup> As a consequence, there is heightened breakdown of ATP and this provides energy for pumping the sodium out of the cell, liberates ADP and inorganic phosphate, and stimulates glycolysis. Because of the accelerated glycolysis, the changes associated with sterile incubation of normal red cells are exaggerated in HS.<sup>85</sup>

The increased  $\text{Na}^+$  flux, however, does not explain the accelerated destruction of HS cells *in vivo*. After splenectomy this defect persists, but the survival of the cells is essentially normal. This observation focused attention on the membrane and this in turn stimulated study of the membrane lipids. It was then found that the total lipid content of HS cells is lower than that of normal cells,<sup>68,88</sup> although the relative proportions of cholesterol and phospholipid as well as the various phospholipids are normal.<sup>45</sup> HS cells were shown to lose membrane lipids excessively during incubation *in vitro*.<sup>57,104</sup> The loss is less pronounced in the HS cells that circulate following splenectomy,<sup>19,88</sup> but the lipid content of such cells still is lower than that of cells from splenectomized normal persons.<sup>19</sup> The lipid depletion has been at-

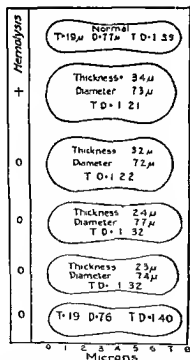


Fig 21-4 Changes in the shape and measurements of the mean erythrocytes of normal blood on the addition of varying amounts of distilled water to the plasma (From Haden,<sup>44</sup> courtesy of the author and American Journal of Medical Sciences)

tributed to the greatly increased metabolic activity required for the accelerated pumping of sodium ions, this being associated with increased phospholipid turnover.<sup>57</sup> With the loss of lipid and, consequently, of surface area, HS red cells lose pliability and plasticity.

In the course of its normal life-span the red corpuscle must traverse slit-like stomas, some of which may be as small as 3  $\mu$ m. To accomplish this it must possess sufficient membrane surface area to undergo the extremes of deformation imposed by the circulation and yet resume its normal biconcave shape. Of the hazards of the circulation, the spleen is the most exacting and if the corpuscle is not easily deformable it may be trapped there.

Well before the membrane defect of the HS cell, as manifested by the sodium leak, was clearly identified and the lipid depletion of the HS membrane was demonstrated, Young et al,<sup>112</sup> as well as others, showed that the spleen serves as a filter for the red cells, and a trap. Sequestered there under conditions of hemoconcentration that remove them from the glucose that is readily available in circulating plasma, these cells, with their increased need for glucose, ultimately break down.

The exact nature of the membrane defect, however, still is obscure. Attention now is being directed to the membrane proteins.<sup>60,104</sup> Study of these proteins has been hampered by a lack of techniques for obtaining them in undenatured form. On the basis of *in vitro* studies it has been reported that there is abnormal aggregation of HS mem-

brane components and that microfilament formation is defective.<sup>60</sup> It has been postulated that membrane proteins of HS cells are genetically altered in such a way as to interfere with their proper conformation, perhaps into fibrils. Possibly as a result of mutations in the microfilamentous proteins of the red cell membrane, the normal plasticity of the red corpuscle is impaired.<sup>58</sup> The membrane protein abnormality may be associated with thiol groups in the membrane, according to electrophoretic studies.<sup>46</sup>

Spherocytosis and neonatal jaundice are found in the deer mouse,<sup>1</sup> but this animal model so far has not been found useful in studying the pathogenesis of HS in man.

## Hereditary Elliptocytosis (HE)

The circulating red cells of nonmammalian vertebrates are oval or elliptical and nucleated. Among the mammals, only camels and llamas have red corpuscles that are oval or elliptical in shape, but their corpuscles are not nucleated. In man, only a small proportion (1 to 15%)<sup>131</sup> of the red corpuscles are oval, but in persons with anemia the number of such cells may increase, especially in those with macrocytic, and, to a smaller extent, in those with microcytic anemias.<sup>131</sup> Oval macrocytes are typical of the megaloblastic anemias (Chapter 14) and oval and elliptical cells are seen in sickle cell anemia, thalassemia, and in hemolytic anemias associated with red cell enzymatic defects. The varieties of oval and elliptical shapes that may be encountered are illustrated in Figure 21-5.

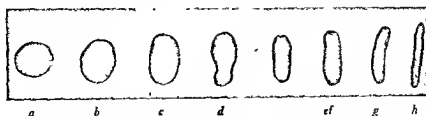


Fig. 21-5 Camera lucida drawings of erythrocytes found in the blood of persons having the elliptical red corpuscle trait. *a* represents an approximately normal cell, *b*, *c*, and *d* represent "oval" forms, *e*, *f*, *g*, and *h* represent "rod" forms. (From Florman and Wintrobe,<sup>131</sup> courtesy of the authors and Bulletin of the Johns Hopkins Hospital)

In patients with the inherited disorder HE, at least more than 25%<sup>122</sup> and usually as many as 50 to 90%<sup>143</sup> of the red cells are rod-shaped or oval (Fig. 21-6).

### Incidence and Genetics

The incidence of HE in the general population is about 0.04%.<sup>122, 161</sup> This anomaly, first noted by Austin Flint<sup>130</sup> and described by Dresbach in 1904,<sup>129</sup> is widespread throughout the world<sup>125, 132, 136, 139, 152, 156</sup> and is transmitted as an autosomal dominant trait.<sup>126, 133, 135</sup> Members of either sex may be affected (Fig. 21-7). Linkage of the gene for elliptocytosis with the Rh blood type has been demonstrated in some families<sup>134, 145</sup> but not in others,<sup>122, 126, 133, 145</sup> and it was suggested that the linked form of elliptocytosis is less likely than the nonlinked variety to be associated with hemolytic anemia.<sup>122, 161</sup> It is difficult to draw a sharp distinction on this basis, however. Data are conflicting regarding the uniformity of hemolytic manifestations in family members,<sup>125, 149, 161</sup> in part perhaps because clear differentiation between compensated hemolytic disease and freedom from hemolysis has not always been made.

HE has been observed in association with G6PD deficiency,<sup>148, 152</sup> glyoxalase II deficiency,<sup>157</sup> thalassemia,<sup>120</sup> HbS, HbC,<sup>160</sup> and hereditary hemorrhagic telangiectasia.<sup>150</sup>



Fig 21-6 Hereditary elliptocytosis (Blood smear, Wright stain  $\times 900$ )

### Clinical Manifestations

In the majority of persons carrying this trait, no clinical manifestations are present; in about 12%, evidence of increased hemolysis is found, according to one estimate.<sup>150</sup> In one family, however, 57% manifested signs of hemolysis at one time or another.<sup>139</sup> Anemia may or may not be noted even when hemolytic disease is present, this being governed by the capacity of the bone marrow to compensate for the accelerated destruction. In some instances the erythrocyte count may be higher than normal, although the amount of hemoglobin and the volume of packed red cells are normal.<sup>131</sup> The hemolytic disorder may be accompanied by signs of chronic hemolysis (Chapter 20) such as reticulocytosis, splenomegaly,<sup>159</sup> leg ulcers,<sup>138</sup> and, occasionally, even skeletal abnormalities.<sup>123</sup> Anemic crises have been observed, possibly related to infections.<sup>128</sup>

### The Blood Picture

Unlike sickle cells, elliptocytes do not change their shape in sealed fresh blood preparations.<sup>131, 145</sup> On the average they are 8.1  $\mu\text{m}$  long and 5.3  $\mu\text{m}$  wide,<sup>131</sup> but they may be as long as 12.2  $\mu\text{m}$  and as narrow as 1.6  $\mu\text{m}$ . Their axial ratio was stated to be 0.78.<sup>143</sup> In one study, MCV ranged from 50 to 76 fl and the MCH 18 to 28 pg with MCHC within normal limits,<sup>131</sup> but others have reported normal values for MCV and MCH.<sup>127, 150</sup>

The number of elliptical forms in the blood of a newborn child gradually increases until the child is three or four months of age, after which their number stabilizes.<sup>136, 137, 138</sup> Hemolysis and hyperbilirubinemia requiring exchange transfusion have been observed in the newborn period; at this time the morphologic picture may more closely resemble pyknocytosis (page 765) than elliptocytosis.<sup>121</sup>

The life span of elliptocytes may be normal or, in hemolytic disease, it is shortened.<sup>146</sup> No morphologic differences have been detected between elliptocytes with normal or shortened life span.

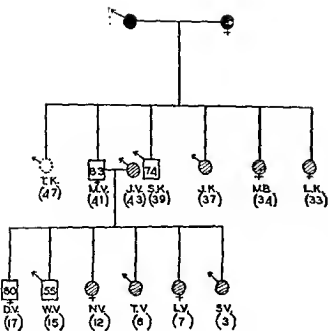


Fig 21-7. Family K (Negro), showing incidence of elliptical cell trait. The open blocks represent subjects in whom many elliptical cells were found. The proportion of such cells is given within the block. A hatched circle represents an individual whose red corpuscles were not elliptical, an open circle indicates those not examined, and a black circle refers to those dead. Each individual's age is shown in parentheses below the initials. (From Florman and Wintrobe,<sup>131</sup> courtesy of the authors and Bulletin of the Johns Hopkins Hospital.)

Osmotic fragility, even after incubation, and autohemolysis usually are normal,<sup>113,135,141,150</sup> but when there is hemolytic activity they have been found to be abnormal.<sup>126,128,135,158</sup> When autohemolysis was increased it was reduced by the addition of glucose or ATP.<sup>135,158</sup> In persons with hemolytic disease, reticulocytes are increased, frequently to levels up to 10%, occasionally higher, and spherocytes may be present. Haptoglobin is decreased.<sup>133,139</sup> Spherocytes may increase in number post-splenectomy, but the number of reticulocytes decreases to normal values.

The reaction to Coombs' test is negative in uncomplicated cases.

## Diagnosis

As mentioned previously, elliptocytes may be present in many forms of anemia but not in the numbers characteristic of HE. Persons harboring this anomaly, however, may develop iron deficiency or other forms of anemia and, as has been noted, this hereditary trait may be associated with other inherited abnormalities. These must be differentiated by the usual methods. Differentiation from HS may not be easy. The diagnosis of HE

is based on the large proportion of elliptocytes present in the blood (page 760).

## Treatment

Sequestration of the red cells in the spleen has been observed<sup>126</sup> and splenectomy has been found to relieve the hemolytic anemia,<sup>124,142,143,146</sup> even though the morphologic abnormality of the red corpuscles is not altered.<sup>143</sup> In fact, the microovalocytes, bizarre-shaped red cells, and red cell fragments that may be found in the blood of patients with hemolysis may increase in number following splenectomy.<sup>159</sup>

## Pathologic Physiology

The factors that determine the anomalous shape are unknown. The nucleated precursors of elliptocytes are round,<sup>131</sup> and even reticulocytes do not show the degree of elliptocytosis seen in mature corpuscles.<sup>155</sup> The erythroblasts with elliptical nuclei, described by Löffler and Hansen,<sup>144</sup> probably represent a different anomaly. The elliptical shape persists in various isotonic solutions, in surviving cells (ghosts) in hypotonic saline solution, and in the plasma of normal individuals.<sup>155</sup>

Radioautographs showed the membrane cholesterol to be concentrated toward the convex surface around the periphery of the cells, i.e., the point of greatest convexity.<sup>147</sup> Electron microscopy and shadow-casting showed the hemoglobin to be aggregated in a bipolar arrangement.<sup>153</sup> No abnormalities in the hemoglobin and no deficiency in glycolytic enzymes have been discovered.<sup>126, 142, 143, 156</sup>

On sterile incubation *in vitro*, a more rapid than normal decline in cellular ATP and 2,3 DPG was reported<sup>135</sup> and a 40% increase in the sodium efflux that is inhibited by ouabain was observed.<sup>154</sup> These findings suggest a membrane permeability defect similar to that of HS, but neither the degree of ATP instability nor the rate of sodium efflux has been found to be correlated with the presence or absence of shortened red cell survival.

No feature that clearly differentiates the asymptomatic<sup>161</sup> and the hemolytic states of HE has been identified. Although persons with the type of elliptocytosis that is linked with the Rh blood group have usually been free of hemolytic disease, this has not always been the case.<sup>126, 142</sup> Neither has the same degree of hemolytic activity been demonstrated in members of the same family. Homozygosity for the trait was suggested as the cause of severe hemolysis in certain patients.<sup>143, 152, 161</sup>

## Acanthocytosis

An acanthocyte is a spherical red corpuscle with several irregularly spaced, large ("thorny") projections that vary in width and length. As discussed in Chapter 13 (page 541) its appearance contrasts with that of another form of spicule cell, the echinocyte or burr cell that possesses regularly spaced spicules, more uniform in size and more numerous than the projections of acanthocytes.<sup>183</sup>

### Abetalipoproteinemia

#### Clinical Manifestations

First recognized in a girl born of parents who were first cousins,<sup>170</sup> abetalipoproteinemia

is characterized by retarded growth; steatorrhea; progressive ataxic neurologic disease involving the posterior columns, the pyramidal tracts, and the cerebellar pathways; and retinitis pigmentosa.<sup>189, 200</sup> The blood contains numerous acanthocytes (Fig. 21-8), but, despite these striking morphologic abnormalities, anemia is absent or mild.<sup>189</sup> The serum lipids are extremely low, causing the serum to appear remarkably transparent. The serum contains no  $\beta$ -lipoprotein (low-density lipoprotein) and the serum cholesterol, triglyceride, and phospholipid levels are lower than normal.<sup>191, 194, 195</sup> The cholesterol content of acanthocytes is either normal<sup>191</sup> or high, but lecithins, the major class of phospholipids in normal human red cells, are decreased,<sup>172, 189, 192</sup> both in relative and absolute amounts, and sphingomyelins are increased.<sup>204</sup> Observed in a number of other instances in the offspring of consanguineous marriages,<sup>170, 175, 189</sup> the syndrome is rare and is thought to be inherited from both parents as an autosomal recessive trait.

Younger red cells in persons with this disorder show minimal or no morphologic distortion, but the degree of cell deformity increases with cell maturation or aging, or both.<sup>192</sup> During 48 hours' incubation the red cells become permeable to hemoglobin more rapidly than do normal cells.<sup>197</sup> This auto-hemolytic pattern is accentuated if the blood is collected in EDTA and maintained at room temperature for 48 to 72 hours. It is inhibited



Fig 21-8. Acanthocytes from a patient with abetalipoproteinemia (Courtesy of Dr Robert S Lees)

by conditions leading to better preservation of intracellular ATP.<sup>197</sup> Small amounts of whole serum, separated low- and high-density lipoproteins, and certain emulsions of plasma lipids inhibit the autohemolysis of acanthocytes.<sup>203</sup> Peroxidative stress increases hemolysis, and substances that prevent lipid peroxidation, such as tocopherol (Chapter 4, page 148), prevent hemolysis.<sup>174,184</sup> Osmotic fragility usually is in the normal range.<sup>197</sup> The *in vivo* life span of the red cells in abetalipoproteinemia may be slightly shortened,<sup>197,204</sup> but this accelerated destruction is intermittent. Slightly increased or normal numbers of reticulocytes are observed.<sup>197</sup>

### Biochemical Defect

The primary biochemical lesion in abetalipoproteinemia may be a selective one, affecting the main apolipoprotein ( $\beta$ -apoprotein, apoLP-ser) of low-density lipoprotein.<sup>179</sup> The  $\beta$ -apoprotein seems to be an obligatory component of chylomicrons as well as very low-density lipoprotein. Thus, the formation of these lipoproteins also is defective and transportation of long-chain fatty acids from the gut or mobilization of triglyceride from the liver cannot take place in normal fashion.<sup>182,185</sup> In contrast to celiac disease, fat absorption alone is impaired and the absorption of other nutrients such as vitamin B<sub>12</sub> is normal,<sup>197</sup> as is cation permeability.<sup>180</sup> Deficiencies of the fat-soluble vitamins A, E, and K may be responsible for at least some of the clinical features of abetalipoproteinemia, but their precise role in the pathogenesis of the retinal and neurologic changes remains to be defined. The morphologic features of the acanthocytes can be reversed *in vitro* by non-ionic detergents,<sup>201</sup> an observation indicating the importance of the plasma lipid and lipoprotein milieu to normal red cell structure and function.

The activity of the serum cholesterol esterifying enzyme, lecithin:cholesterol acyltransferase (LCAT), has been found to be reduced to approximately 40% of normal in persons with abetalipoproteinemia,<sup>172</sup> as well as in most patients with liver disease who

have either target cells or spur cells.<sup>171</sup> This implies that the enzyme plays an important role in the metabolism of unesterified cholesterol and lecithin in serum lipoproteins, but its role in the pathogenesis of abetalipoproteinemia still is uncertain.

A number of patients have been reported in whom variant syndromes have been noted.<sup>178,186</sup> These included patients who were affected with acanthocytosis and progressive neurologic impairment and yet normal lipoproteins were found. In others, diminished but detectable beta-lipoproteins were present.

### Diagnosis

Abetalipoproteinemia is suspected on the basis of clinical findings, acanthocytosis, and markedly diminished serum cholesterol and triglyceride concentrations. The diagnosis is confirmed by absence of beta-lipoprotein in plasma and the characteristic morphologic appearance of a small intestinal biopsy specimen; the mucosal cells are filled with lipid droplets, especially at their villous tips. Fat droplets are virtually absent in the intercellular space and lacteals.

Abetalipoproteinemia should not be confused with familial alpha- (high-density) lipoprotein deficiency (Tangier disease), in which hypocholesterolemia (cholesterol concentration <100 mg/dl) usually also occurs and enlargement of the spleen, liver, and lymph nodes may be present but acanthocytosis is not noted.<sup>177</sup> In alpha-lipoprotein deficiency, increased proportions of phosphatidyl choline and decreased proportions of sphingomyelin in both the plasma and the red corpuscles have been reported.<sup>196</sup>

As discussed in Chapter 19 (page 706), patients with hepatocellular disease may have hemolytic anemia associated with a high proportion of cells resembling the acanthocytes of abetalipoproteinemia ("spur cells").<sup>171</sup> This is attributable to the markedly increased cholesterol content and cholesterol-phospholipid ratio of the cells. The various forms of spicule distortion of red cells are discussed in Chapter 13.



## Treatment and Prognosis

Although there is no definitive therapy for patients with abetalipoproteinemia, and some of the patients have succumbed at an early age, treatment with high doses of vitamins A and E has been given in the hope that the abnormalities in retinal and neurologic function will be ameliorated.

## Other Disorders Involving the Red Cell Membrane

### Stomatocytosis

✓ A linear area of central pallor in red corpuscles as seen in stained films characterizes the "stomatocyte." The slit-like appearance contrasts with the normal circular area of central pallor of red cells. Stomatocytes seem to be uniconcave, and in wet preparations they have a bowl-like appearance. They can be seen occasionally in blood films and occur as a transient phenomenon in persons with acute alcoholism, but in several reported patients with inherited disease they were present consistently, in large numbers, and in association with mild or severe hemolytic anemia (see Fig. 13-2, page 545).

The mother and daughter reported by Lock and his associates<sup>215</sup> manifested symptoms resembling HS, in that they had hemolytic anemia of moderate degree, associated with greatly increased osmotic fragility. Their disease differed from HS in that stomatocytes rather than spherocytes were found in the blood and splenectomy was of no benefit. The survival of their red cells was very short, even after splenectomy, and was even shorter when their cells were transfused into normal recipients with intact spleens. Similar cases have been reported by others.<sup>217,219</sup> Differing somewhat from these cases are those in a 6 month old child<sup>226</sup> and in a father, son, and grandson of one family.<sup>221</sup> These patients had a mild hemolytic disorder characterized by stomatocytes that contained very high levels of sodium and low levels of potassium. The red cells were found

to be 20 to 40 times more permeable to  $\text{Na}^+$  and  $\text{K}^+$  than normal. There was no evidence of abnormality in the parents of the 6 month old child, but in the family studied by Oski and his associates<sup>221</sup> the pattern of inheritance resembled that of HS and in these patients, as in those with HS, osmotic fragility was increased and splenectomy was beneficial. It was observed that as the red cells aged they became more permeable to  $\text{Na}^+$ . The observations in these cases suggested an abnormality in the functioning of the red cell cation pump.

From Australia, mild hemolytic anemia with stomatocytosis and normal osmotic fragility has been reported in some Mediterranean migrants,<sup>211,213</sup> but, in others, stomatocytosis was present in the absence of anemia or reticulocytosis.<sup>220</sup> In none of the subjects of Mediterranean origin were abnormalities of red cell metabolism or of hemoglobin found<sup>211</sup> nor was there evidence of electrolyte changes<sup>213</sup> or abnormal osmotic fragility.<sup>211</sup>

Still another form of hereditary hemolytic anemia associated with stomatocytosis has been described in members of a Swiss-German family.<sup>218</sup> In these subjects, osmotic fragility was decreased, and red cell  $\text{Na}^+$  content,  $\text{K}^+$  leak, and sodium pump rates were increased. Autohemolysis was type I. The parents of these patients were consanguineous and inheritance was autosomal dominant.

In still another family, elliptically shaped erythrocytes with one or more transverse slitlike areas of decreased density and with increased permeability to  $\text{Na}^+$  and  $\text{K}^+$  were associated with no anemia or evidence of hemolytic disease.<sup>212</sup> In vitro erythrocyte glucose consumption was 60% greater than that of normal controls. Thus it appears that erythrocytes can compensate for increased cation permeability without any ill effects being apparent.

### Hereditary Nonspherocytic Hemolytic Anemia with Altered Erythrocyte Phospholipid Composition

In a family from the Dominican Republic a dominantly transmitted mild hemolytic

anemia was observed in which osmotic fragility was decreased while autohemolysis was slightly increased but was corrected to nearly normal by the addition of glucose. However, unique morphologic characteristics were not observed.<sup>214</sup> The life span of isologous, but not homologous, transfused erythrocytes was reduced. An absolute increase in phosphatidyl choline (lecithin) (PC) in the red cells was demonstrated, but there was no detectable abnormality of plasma lipids. The accumulation of PC in these patients' cells has been shown to be due to inhibition of the transfer of actively acquired esterified membrane fatty acid from PC to phosphatidylethanolamine (PE) prior to its return to the plasma<sup>223,225</sup> (see Chapter 3, page 97). This is associated with increased cation permeability and with increased glycolysis because of the higher energy requirement for operation of the cation pump<sup>224,225</sup>; it results in increased vulnerability of the red cells to stress.

### LCAT Deficiency

Total absence of lecithin:cholesterol acyltransferase associated with normocytic anemia and attributed to slight hemolysis and reduced compensatory increase in erythropoiesis has been described in three sisters.<sup>178</sup> The erythrocyte content of cholesterol was markedly increased and PE and sphingomyelin were decreased. In contrast to hereditary acanthocytosis, in which LCAT is reduced but not absent (page 763), numerous target cells were seen in the blood smear.

### "Infantile Pyknocytosis"

"Infantile pyknocytosis" refers to the condition in which distorted, burr-like red corpuscles, probably caused by extracorporeal factors,<sup>210</sup> are found in association with a clinical picture consistent with that of erythroblastosis fetalis in some instances, and that of anemia and jaundice developing in the first few weeks of life in others. No cause has been discovered and the usual management of neonatal hemolytic disease has been successful in this disorder.

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## *Hereditary Hemolytic Anemias Associated Mainly with Abnormalities in the Glycolytic Metabolic Pathway of Erythrocytes<sup>60,91</sup>*

### **Pyruvate Kinase Deficiency Other Enzyme Deficiencies**

**F**OLLOWING the recognition of hereditary spherocytosis (HS) (Chapter 21) as a specific entity it came to be appreciated<sup>28</sup> that cases of congenital hemolytic anemia which do not conform to this classic pattern are sometimes encountered. As described elsewhere (Chapter 20), under the term "congenital nonspherocytic hemolytic anemia," a number of investigators drew attention to the fact that in certain of these subjects spherocytosis and increased osmotic fragility of fresh blood are not found, hemoglobin abnormality cannot be demonstrated, and splenectomy is only partially beneficial, if at all.<sup>19</sup> The autohemolysis test (page 735) of Selwyn and Dacie<sup>57</sup> served additionally to distinguish these cases, categorizing two types; furthermore, by revealing that the addition of glucose to the incubation mixture failed to prevent autohemolysis, in contrast to the effect in HS, the possibility arose that impaired glycolysis might be responsible for some of the observed differences.<sup>33,53,90</sup> The discovery of

glucose-6-phosphate dehydrogenase deficiency (Chapter 23) and the subsequent demonstration of red cell pyruvate kinase deficiency in association with hereditary nonspherocytic hemolytic anemia<sup>63</sup> initiated a series of studies that have since revealed a number of other hereditary enzymatic deficiencies in erythrocytes, most of which are associated with various degrees of hemolytic anemia. Those involving the glycolytic pathway will be discussed here. Those related to the hexose monophosphate shunt and glutathione metabolism, especially glucose-6-phosphate dehydrogenase deficiency, will be considered in Chapter 23.

### **Pyruvate Kinase (PK) Deficiency**

Of the enzymatic deficiencies involving the Embden-Meyerhof pathway, PK deficiency is the most common and can serve as the prototype for the remainder, which are quite rare. A review published in 1971 listed 135 well-documented cases, including some previously described as instances of Dacie's Type II nonspherocytic hemolytic anemia.<sup>60</sup> Members of both sexes are equally affected. Al-

Table 22-1. Some Features of Certain Inherited Red Blood Cell Enzyme Deficiency Disorders

Enzyme Deficiency* <sup>1, 114</sup>	Mode of Inheritance	Severity of Anemia	Hemolysis after 24 to 48 Hr Incubation at 37°C			ATP	Autohemolysis Type	WBC Enzyme Concentration	Associated Disorders
			no additive	glucose					
Glycolytic pathway									
HK <sup>15, 16, 117</sup>	AR	Mild to severe	++ or +++	+		+	I	N A?	Probably none
GP1 <sup>15, 16, 18, 105</sup>	AR	Moderate to severe	++ or +++	+		+	I	Low, also low in plasma	None
PFK <sup>11, 12, 120</sup>	AR?	Mild	+++					N (low in muscle)	Myopathy
TP1 <sup>16</sup>	AR	Severe	++	0		0	As in HS	Low (also low in muscle, serum CSF)	Neurologic myocardial infectious
2,3 DPGM <sup>11, 16, 107</sup>	AR	Severe to moderate	+++	+		+	I		None
PGK <sup>11, 116</sup>	X-linked	Severe to moderate	++	0		+	I	Low	Mental retardation
PK <sup>16</sup>	AR	Mild to severe	+++	+++		+	II	N	None
LDH <sup>100</sup>	AR	None							
Glutathione metabolism									
G6PD (Chapter 23)	X-linked	None to severe	++ or +++	+		+	I	N A	Sensitivity to oxidant drugs, etc
6PGD (Chapter 23)	AR	None	+++	+		+	I		
GR (Chapter 23)	AD?	?	+++	++		0	II	N	
GSH-Px (Chapter 23)	AR	Mild to moderate?							Drug sensitivity?
Glutathyl cysteine synthetase	AR	Mild	+	+					
GSH synthetase	AR	Mild							
Other RBC enzymes									
AK <sup>110</sup>	AR	Moderate?							
RPK <sup>111</sup>	AR?	Moderate	+++	++		0	II		
ATPase <sup>117, 10</sup>	AD	Moderate							

The abbreviations for the various enzymes are explained in the text

AR means autosomal recessive AD autosomal dominant; N means normal, A abnormal; HS is hereditary spherocytosis. References are given in the first column, except for enzymes involved in glutathione metabolism, which will be found in Chapter 23.

though most of the cases have been found in persons of Northern European origin,<sup>8</sup> the disorder has been described in several Italians,<sup>14</sup> in a Syrian, a Spanish infant, a Mexican child, in American Negroes, in Japanese,<sup>37</sup> and in Chinese.<sup>60</sup> A high frequency was identified in an inbred Amish commune<sup>12</sup> and in Chinese newborn.<sup>22</sup> As additional cases are reported it is becoming apparent that PK deficiency is a somewhat polymorphous disorder.

### Clinical Manifestations

The characteristic features of chronic hemolytic anemias, outlined in Chapter 20, are present: anemia of varying degree, jaundice, splenomegaly, episodes of dark urine, increased incidence of gallstones.<sup>60</sup> The hemolytic process may be severe<sup>11,40</sup> and may be aggravated by intercurrent infection or other stressful situations.<sup>32,33,49,63</sup> Aplastic crises have been observed.<sup>32,44</sup> Remarkable is the variation in the severity of the disease,<sup>60,63,67</sup> ranging from pronounced neonatal jaundice requiring exchange or multiple transfusions<sup>8,22</sup> to a fully compensated hemolytic process in adults.<sup>44,63</sup> PK deficiency generally is a more serious disease than HS for two reasons, i.e., the greater degree of clinical severity in many PK-deficient subjects and the absence of unequivocal benefit from splenectomy.

In most of the patients with PK deficiency, anemia or jaundice have been noted in infancy or early childhood but in some the disease has not been detected until adulthood, even not until late in life.<sup>41</sup> Although survival to adult life is common, in the severe form found in Amish kindreds, death before the age of 3 or 4 years was likely unless splenectomy was performed.<sup>11,12</sup>

General development usually is normal, but may be impaired, and bone changes associated with hyperplastic bone marrow, such as those seen in other hereditary chronic hemolytic anemias (Chapters 25, 26), may result in prominence of the frontal cranial eminences.<sup>1,11,63</sup> Splenomegaly is slight to moderate in degree. Chronic leg ulcers are

rare.<sup>60,63</sup> Pregnancy has been tolerated without unusual complications.<sup>33,37</sup>

### Laboratory Findings

#### *The Blood*

Although the degree of anemia varies widely, hemoglobin levels of 6 to 12 g/dl and packed cell volumes of 0.17 to 0.37 l/l have been most common.<sup>60</sup> A moderate degree of spontaneous fluctuation may be observed. Extremely low levels have been reported during the first few years of life with stabilization at higher levels later.

Macrocytosis of slight to moderate degree is noted in association with reticulocytosis. The percentage of reticulocytes ranges from 2.5 to 15.0, but values as high as 56.0% have been reported after splenectomy.<sup>60</sup> There is moderate polychromatophilia, as well as variable numbers of nucleated red cells. Although only slight aniso- or poikilocytosis are usually present, occasional, irregularly contracted erythrocytes with irregular borders ("spicules"<sup>41</sup>), tailed poikilocytes, or elongated forms have been described,<sup>33,37</sup> and, in infants and young children, cells resembling acanthocytes have been reported.<sup>49</sup> The leukocyte and platelet counts are normal or slightly increased.

*Osmotic fragility* of fresh red cells is normal, but on incubation different degrees of increased fragility are found.<sup>11,37,44,63</sup> In the autohemolysis test, increased hemolysis usually is observed after 48 hours of sterile incubation and this is not reduced by glucose but is correctible by ATP (Type II)<sup>23,24,63</sup>; however, in mildly affected individuals this test may give normal findings and a few instances of Type I autohemolysis were reported.<sup>2,15,37,60</sup>

#### *Other Laboratory Findings*

Serum bilirubin is moderately elevated, plasma hemoglobin is not increased, but serum haptoglobin may be decreased or absent.<sup>11,44,48</sup> Fecal urobilinogen is increased. Serum iron is normal or slightly increased



and total iron-binding capacity is normal. Reactions to the Coombs' test are negative and Hb F and Hb A<sub>2</sub> concentrations are normal.

Ferrokinetic studies indicate active effective erythropoiesis and there is moderate to severe shortening of the life span of the red cells (Fig. 20-2),<sup>42</sup> although this is quite variable.<sup>60</sup> The significance of <sup>51</sup>Cr half-time measurements in this disorder may well be questioned since the cells available for tagging are presumably those that have survived the early rapid attrition of the most severely affected erythrocytes.<sup>60</sup> As in other hemolytic disorders, erythroid hyperplasia is found in the bone marrow.

### Pathologic Physiology

Erythrocyte pyruvate kinase is an enzyme that may have a molecular weight of 225,400.<sup>17</sup> It catalyzes the regeneration of ATP from ADP and provides the pyruvate for the subsequent conversion to lactate, as follows: phosphoenolpyruvate + ADP  $\xrightarrow{\text{PK}}$  pyruvate + ATP. The precise pathogenetic mechanism resulting in the ultimate destruction of PK-deficient cells and the consequent hemolytic anemia still is obscure. There is no close correlation between the enzyme level and the apparent severity of the hemolytic anemia.<sup>23</sup> Various possibilities have received attention.

It is from ATP that erythrocytes derive their energy for vital functions such as maintenance of cationic gradients. Consequently, it is reasonable to assume that the impairment in ATP regeneration in PK deficiency accounts for their diminished life span.<sup>40</sup> Rather than gaining sodium, as occurs in HS (Chapter 21), PK-deficient erythrocytes lose potassium.<sup>33,42</sup> This probably explains the corpuscular distortions that have been described in the blood and even makes the cells vulnerable to destruction in the liver, as well as the spleen.

Several observers have noted increased concentration of reticulocytes in the spleen of patients with PK deficiency.<sup>11,40,42</sup> Theo-

retically, reticulocytes should be less vulnerable than mature erythrocytes to deficient PK activity since they possess mitochondria and can utilize mitochondrial oxidative phosphorylation to maintain ATP levels.<sup>33</sup> However, PK-deficient reticulocytes with particularly low levels of PK activity are especially reliant upon oxidative phosphorylation in the mitochondria. In the spleen such cells may be subjected to metabolic factors, such as low pH, low P<sub>O<sub>2</sub></sub>, and low glucose level, that may handicap reticulocyte mitochondrial metabolism.<sup>36</sup> With the consequent rapid fall of ATP level, selective sequestration of the defective reticulocytes would occur. Various phases of reticulocyte phagocytosis by the cordal macrophages of the spleen have been observed.<sup>35</sup> Some variation in the degree of impairment of PK activity in erythrocytes, whether they be reticulated or mature, has been postulated.

Whether PK deficiency is the primary defect in this disorder or is only indicative of another abnormality, either of the red cell membrane or of its metabolic processes,<sup>61</sup> is unknown. Abnormal accumulations of glycolytic intermediates have been found in the red corpuscles, especially 2,3 DPG<sup>23</sup> and phosphoenolpyruvate,<sup>31</sup> but these can be explained by the fact that the metabolic defect is late in the glycolytic pathway and such accumulations might be expected (Fig. 3-14, page 103).

### Genetics

Although rare exceptions have been reported,<sup>30</sup> it is generally accepted that erythrocyte PK deficiency is inherited as an autosomal recessive trait. Only the red cells are affected. Leukocytes, for example, are not affected.<sup>63</sup> Only individuals homozygous for this defect manifest clinical disease.<sup>60,63</sup> In homozygotes, erythrocyte PK activity has been found to be in the range of 5 to 25% of normal mean values; erythrocytes of heterozygotes generally have about half normal PK activity. The PK of human red cells is known to exist normally in at least three isozymic forms.<sup>6</sup> As with other enzymes, there are mutant forms of PK<sup>7,31,53</sup> which,

though capable of catalyzing the same biochemical reactions, have abnormal kinetic properties that result in impaired catalytic efficiency at the low substrate concentrations found in erythrocytes.<sup>9</sup>

The observations of a number of investigators indicate that PK deficiency is genetically heterogeneous.<sup>47,51</sup> Extreme intrafamilial differences between PK phenotypes have been observed.<sup>67</sup> Double heterozygosity for mutant PK isozymes and deficient normal enzyme probably explain seeming normality in one or the other of the parents of affected individuals.<sup>13,52,53,60</sup> This would account for at least some of the hypotheses, proposing autosomal dominant inheritance of PK deficiency, that were based on failure to demonstrate quantitative deficiency of normal PK.<sup>2,15,66</sup>

## Diagnosis

The diagnosis depends first on recognition by the usual criteria (Chapter 20) of the fact that one is dealing with a hereditary hemolytic anemia of chronic nature that differs from HS, eg, absence of spherocytosis, normal osmotic fragility of fresh blood, and a type II autohemolysis pattern (page 735). A history may be obtained of severe jaundice and even exchange transfusion in the neonatal period without evidence of fetomaternal Rh or ABO incompatibility. The history, however, may not necessarily reveal the hereditary nature of the disorder or even chronicity and, consequently, the possibility of acquired hemolytic processes must be excluded by history and negative reactions to the Coombs' test and to tests for paroxysmal nocturnal hemoglobinuria (Chapter 29). In a simple screening test for PK activity,<sup>5</sup> the PK reaction is coupled to the NADH-dependent conversion of pyruvate to lactate. Since NADH fluoresces when irradiated with long-wave ultraviolet light and NAD does not, the reaction can be monitored visually. Phosphoenolpyruvate, NADH, and LDH are added to blood, which supplies PK. As pyruvate is generated, NADH is utilized. In PK deficiency the NADH fluorescence persists

even for 45 to 60 minutes, in contrast to the disappearance of fluorescence within 15 minutes when normal blood is used. False results can be expected, however, if the patient has been transfused recently.

Whenever possible, an abnormal result should be confirmed by quantitative assays.<sup>59</sup> Studies of the kinetics, stability, and other properties of the enzyme may be required in order to recognize kinetically aberrant and mutant isozymes. Since the ratio of the PK activity of leukocytes to erythrocytes is 300:1, efficient separation of these cells before measurements are made is essential.<sup>69</sup> Normally values for males and females are similar, but the normal range for red cell PK activity is quite wide and the lower limits of normal have been difficult to define.<sup>60</sup> PK activity is increased in cord blood and in reticulocytes. Subnormal PK values have been observed only in the acute leukemias<sup>9,38,60</sup> and possibly also in refractory or sideroblastic anemia.<sup>9</sup>

## Treatment

There is no specific therapy. The usual hematopoietic agents and steroids are not effective. The anemia may sometimes be of sufficient severity to require blood transfusion, but this procedure is best avoided, if possible, not only because of the immediate hazards of transfusion but also because of the long-term possibilities of iron overload. Improvement has been observed following splenectomy, especially in infants and young children with severe disease.<sup>32,33,42,45,49,60</sup> This can be explained by the exceptional vulnerability of some reticulocytes to splenic sequestration,<sup>36,42</sup> discussed earlier. In the severest forms of PK deficiency, splenectomy seems to have been necessary to permit survival.<sup>11</sup> In some instances, as an affected child grew older, anemia diminished in severity sufficiently to decrease or eliminate the need for blood transfusion. Even osseous defects were found to regress.<sup>11</sup>

Splenectomy, however, is an imperfect form of therapy. An appreciable degree of hemolysis persists in all subjects, and hemo-

lytic<sup>33</sup> or aplastic<sup>32,41</sup> crises have occurred in splenectomized patients. After a period of improvement postsurgically, a gradual decrease in hemoglobin levels may take place.<sup>44</sup> The persistently impaired red cell survival can be attributed to continued, although slower, destruction in the liver.<sup>33,42</sup> Folic acid supplementation (2 to 5 mg daily, given by mouth) has been used in the hope of preventing complicating megaloblastic anemia.

In families afflicted with the disorder, newborns should be checked for kernicterus and exchange transfusion performed when necessary.

### Prognosis

As mentioned earlier, intercurrent infections and other stressful situations may be associated with exacerbations, hemolytic and aplastic crises may occur, and cholelithiasis may develop. Although the degree of severity of the disease varies greatly, survival into adulthood is common.

## Other Enzyme Deficiencies<sup>111</sup>

As mentioned previously, PK deficiency serves as a prototype for deficiencies of certain other enzymes that participate in the glycolytic metabolic pathway of the erythrocyte. In general, when associated with anemia the clinical manifestations of such defects are those of a hereditary nonspherocytic hemolytic anemia. They resemble PK deficiency in many respects, but there are two important exceptions to this generalization. First, in contrast to the autosomal mode of inheritance that characterizes PK deficiency and most of the other glycolytic defects, phosphoglycerate kinase (PGK) deficiency is X-linked. Second, although in a number of instances the enzyme deficiency affects only erythrocytes, or at least significant ill effects are related only to the erythrocyte deficiency, in glucose phosphate isomerase (GPI) deficiency leukocytes also are involved and in triosephosphate

isomerase (TPI) deficiency a great many tissues are affected, resulting in widespread clinical manifestations, especially in the nervous system.

In contrast to PK deficiency, type I auto-hemolysis<sup>57</sup> (moderate increase in hemolysis only partially prevented by the addition of glucose) has been described in hexokinase (HK) deficiency, GPI deficiency, and PGK deficiency. In TPI deficiency, both glucose and ATP protect against hemolysis, as in HS.<sup>114</sup> Specific assay methods are required if the deficiency is to be identified precisely. Such assays are available only in laboratories concerned with research in this field.<sup>9,60,114</sup>

In the following paragraphs the main features of these enzyme deficiency states will be presented, mainly in the order in which they participate in the glycolytic pathway (Fig. 22-1).

### Hexokinase (HK) Deficiency

This enzyme serves in the first step of the glycolytic sequence (Chapter 3). Normally, HK activity is considerably greater in reticulocytes than in mature cells, and this disparity between young and old cells is greater than is found in any of the other glycolytic enzymatic activities. However, in the five month old child with hemolytic anemia reported by Valentine et al,<sup>117</sup> even the HK activity of the patient's reticulocyte-rich blood was lower than that of normal blood. Her reticulocytes were "old before their time."<sup>117</sup> Splenectomy ameliorated the hemolytic process. Peculiarly, a male sibling who gave no evidence of hemolysis had red cell HK activity lower than the patient's, but this may have been explained by the fact that his cells were mainly mature rather than reticulocytes. Hemolytic syndromes also have been reported in two unrelated individuals,<sup>93</sup> as well as in a family in which the father and son exhibited an erythrocytic HK variant that, in this family, was demonstrated also in the leukocytes.<sup>104</sup> Splenectomy in the son was beneficial. In yet another family, panmyelopathy and multiple congenital anomalies of

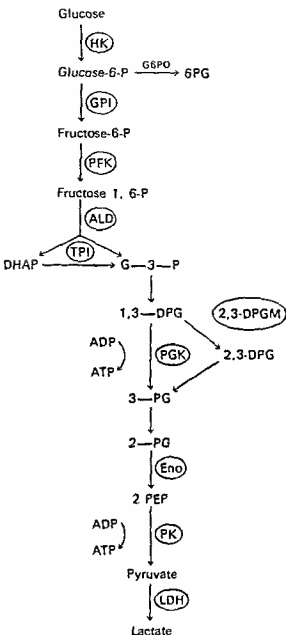


Fig 22-1. Abbreviated diagram of pathway for the metabolic breakdown of glucose in the red corpuscle to show the position of the various enzymes (circled) that have been found deficient in the cases discussed. A more complete diagram is shown in Figure 3-14.

the Fanconi syndrome were associated with diminished HK activity,<sup>97</sup> but the significance of this family in relation to the above cases is obscure. The observed changes may have been the result of the marked chromosomal aberrations that were described.

### Glucose-Phosphate Isomerase (GPI) Deficiency

Since the first reported case of GPI deficiency,<sup>76</sup> a number of additional ones have been studied.<sup>79,103,105,114</sup> This deficiency is characterized by moderately severe hemolytic anemia, partially relieved by splenectomy.<sup>76,105</sup> Selective reticulocyte destruction in the spleen has been reported.<sup>98</sup> In a large proportion of cases the leukocytes shared the deficiency, but no evidence of dysfunction or of increased susceptibility to infection was found. Evidence of synthesis of a qualitatively changed subunit of the GPI molecule that is associated with faster inactivation of the enzyme *in vivo* was presented in one case.<sup>75</sup> Electrophoretic and kinetic studies indicate that GPI deficiency is heterogeneous,<sup>114</sup> and a number of enzyme variants have been noted.<sup>79,101</sup> A method for rapid detection of red cell GPI deficiency based on the appearance of fluorescence caused by TPNH has been described.<sup>78</sup>

### Phosphofructokinase (PFK) Deficiency

PFK deficiency has been reported to be associated with a severe myopathy (sometimes referred to as Type VII glycogen storage disease) together with nonspherocytic hemolytic disease.<sup>112</sup> Two cases have been reported in which the patients did not have muscle dysfunction.<sup>101,120</sup>

### Triosephosphate Isomerase (TPI) Deficiency

TPI deficiency involves not only erythrocytes but also leukocytes, spinal fluid, muscle and cultured skin fibroblasts, and perhaps all tissues.<sup>106,114</sup> Consequently, in addition to severe hemolytic anemia, severe and usually progressive neurologic disease is present and frequent infections and myocardial disease also may result. The gene governing TPI production may reside on the short arm of the number 5 human chromosome.<sup>109</sup> In a highly consanguineous kindred, TPI deficiency was associated with G6PD deficiency

and with the sickle cell trait in various combinations<sup>116</sup>

### 2,3 Diphosphoglyceromutase (2,3 DPGM) Deficiency

2,3 DPGM deficiency has been observed in presumed heterozygotes for this defect,<sup>81,86</sup> and one instance of severe hemolytic anemia in a child both of whose parents showed 50% of normal enzyme activity has been reported.<sup>107</sup> Since the product of the 2,3 DPGM reaction is 2,3 DPG, which plays such a significant role in oxygen affinity (Chapter 3), this type of deficiency is of unusual physiologic interest.

### Phosphoglycerate Kinase (PGK) Deficiency

PGK deficiency also is of special interest because, as in the PK reaction, PGK serves in an ATP regenerating step. In addition, like glucose-6-phosphate dehydrogenase (G6PD) deficiency (Chapter 23), PGK deficiency appears to be X-chromosome linked.<sup>118</sup> Hemolytic anemia associated with PGK deficiency in erythrocytes and leukocytes and with mild mental retardation was described in two male children of a large Chinese kindred and some of the female family members were mildly affected.<sup>118</sup> Similar findings were reported in a Japanese child, except that leukocyte enzyme determinations had not been carried out.<sup>102</sup> One instance of life-long hemolytic anemia in a female subject whose pedigree could not be studied also has been reported.<sup>94</sup>

### Lactic Dehydrogenase (LDH) Deficiency

Severe erythrocyte LDH deficiency without overt evidence of a hemolytic disorder has been reported in an individual who was homozygous for an autosomal, recessively transmitted, hereditary deficiency of a particular subunit (the H-subunit) of LDH.<sup>100</sup>

### Enolase and Aldolase Deficiencies

Chronic hemolytic anemia associated with erythrocyte *enolase* deficiency was reported in

two women who were sisters. In one of these subjects the condition was exacerbated by ingestion of nitrofurantoin.<sup>110</sup> Red cell *aldolase* deficiency has been described in a four year old child with chronic hemolytic anemia, hepatosplenomegaly, and mild mental retardation.<sup>77</sup>

### Deficiencies of Enzymes Not Functioning in the Glycolytic Cycle

In addition to those discussed in the chapter that follows (Chapter 23), in rare instances deficiencies of enzymes not functioning in the glycolytic cycle of the erythrocyte have been reported to be associated with hemolytic anemia. However, the relation of the enzyme deficiency to the anemia has not been clearly established. This comment applies to the reported cases of *adenylate kinase (AK)* deficiency,<sup>90</sup> one of which was associated with G6PD deficiency,<sup>111</sup> and also to *ribosephosphate pyrophosphokinase (RPK)* deficiency. In three unrelated kindreds,<sup>115,119</sup> chronic hemolytic anemia, moderate in degree, with reticulocytosis of about 10%, type II auto-hemolysis, and unusually prominent basophilic stippling of the red cells was associated with RPK deficiency. The levels of red cell glutathione and adenine nucleotides were increased. Although the deficiency was clearly hereditary in nature and was presumed to be transmitted as an autosomal recessive trait, a heterozygous carrier state could not be demonstrated in any of the family members. Whether the enzyme deficiency caused the hemolysis or represented an epiphenomenon was not firmly established.

In the subjects with RPK deficiency, ATP levels were increased. In two other inherited "*high ATP syndromes*," hemolysis was not present.<sup>82,121</sup> In a fourth variety,<sup>84</sup> however, chronic hemolytic anemia was present, but RPK measurements were not reported. In certain other cases, *low ATP* levels were observed in association with severe hemolytic anemia. These, however, were thought most likely to be related to increased catabolism of adenine nucleotides as a consequence of markedly increased utilization of ATP.<sup>99</sup>

## Adenosine Triphosphatase (ATPase) Deficiency

The stroma of the erythrocyte contains two types of ATPase, one of which requires  $\text{Na}^+$  and  $\text{K}^+$  for activation, is involved in ion transport, and can be completely inhibited by cardiac glycosides (page 100), whereas the other plays no role in ion transport and is insensitive to glycosides. Deficiency of the first type of ATPase has been described in a family, some of the members of which had nonspherocytic hemolytic anemia of moderate degree.<sup>60</sup> Dominant inheritance with incomplete penetrance was postulated. One additional case of a presumably similar disorder has been reported.<sup>87</sup>

## Glyceraldehyde-3-Phosphate Dehydrogenase (G3PD) Deficiency

The evidence regarding the association of hemolytic disease with G3PD deficiency and 2,3 DPG phosphatase deficiency is meager and unconvincing.<sup>114</sup>

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# *Glucose-6-Phosphate Dehydrogenase Deficiency and Related Deficiencies Involving the Pentose Phosphate Pathway and Glutathione Metabolism*

## **G6PD Deficiency**

### **Genetics**

### **Incidence and Geographic Distribution**

### **The Enzyme and Its Variants**

### **Mechanisms of Hemolysis**

### **Clinical Manifestations**

### **Diagnosis and Differential Diagnosis**

### **Treatment**

### **Course and Prognosis**

### **Importance of Screening for G6PD Deficiency**

## **Other Deficiencies Involving GSH Metabolism**

### **6-Phosphogluconic Dehydrogenase Deficiency**

### **Glutathione Reductase Deficiency**

### **Glutathione Peroxidase Deficiency**

### **Glutathione Deficiency**

## **G6PD Deficiency**

Glucose-6-phosphate dehydrogenase (G6PD) catalyses the initial step in the pentose phosphate pathway (PPP) (hexose monophosphate [HMP] oxidation pathway) of carbohydrate metabolism, causing reduc-

tion of NADP to NADPH. As discussed in Chapter 3 and illustrated in Figure 23-1, this enzyme plays a most important role in the red blood corpuscle because this corpuscle, having been deprived of an aerobic oxidative pathway by the loss of its mitochondria, depends on the PP oxidation pathway for the generation of NADPH. NADPH is required as a cofactor for red cell glutathione reductase to maintain glutathione (GSH) in the reduced state; reduced GSH, in turn, is necessary (1) for maintaining sulfhydryl groups within the red cell and perhaps in the red cell membrane, and (2), in conjunction with glutathione peroxidase, for the detoxification of peroxide.

The importance of G6PD for the normal metabolism and integrity of red blood corpuscles came to be recognized as the result of the investigation of the unique sensitivity of some individuals to the 8-aminoquinoline antimalarial compound, primaquine. As is outlined later in this chapter (page 785), this showed that the observed sensitivity was due to deficiency of the red cell enzyme G6PD. The original observations were made in



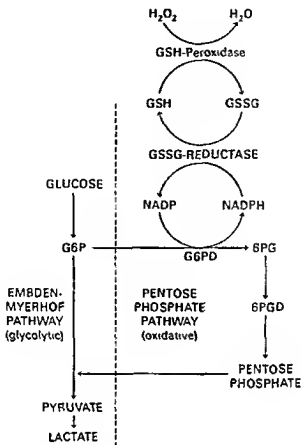


Fig 23-1 The role of G6PD in maintaining glutathione in the reduced state through the reduction of NADP to NADPH thereby making possible the reduction of oxidized glutathione (GSSG) and the role of GSH, in conjunction with GSH peroxidase in the detoxification of peroxide

American Negroes, but G6PD deficiency was later recognized in individuals of Mediterranean ancestry and ultimately in persons living in many parts of the world. Furthermore, it was noted that, whereas, in the subjects first studied, no clinical manifestations were present in the absence of exposure to a drug that caused oxidant stress, in other individuals a more severe degree of G6PD deficiency was present. For example, some subjects with hereditary nonspherocytic hemolytic anemia unrelated to drug ingestion were found to be deficient in G6PD, and still other clinical syndromes such as hemolytic anemia occurring during infection, favism, and neonatal jaundice also were found to be associated with G6PD deficiency. In addition, it is now clear that G6PD "deficiency" is not the con-

sequence of an absence or decreased amount of the enzyme, but is due to the presence of an abnormal, mutant enzyme with abnormal properties. Different variants, with different properties, appear to account for the observed clinical variability. By now, more than 80 such variants have been described. Thus the scientific investigation of sensitivity to an antimalarial compound led to the discovery and clarified the understanding of disorders which now are considered as probably affecting more than 100 million males throughout the world.

#### Genetics

The gene determining the structure of G6PD is carried on the X chromosome.<sup>23,52</sup>

Consequently, the defect is fully expressed in affected males. It cannot be transmitted from father to son, only from mother to son. Female heterozygotes for G6PD deficiency have two populations of cells—normal cells and G6PD-deficient cells. Although the average G6PD activity of a group of females heterozygous for G6PD deficiency is about half that found in normal males, the expression of the defect in individual female heterozygotes varies greatly<sup>48,89</sup> because X-inactivation appears to be a random process and may affect many more paternally derived X chromosomes than maternally derived ones, or vice versa.<sup>9</sup>

### Incidence and Geographic Distribution<sup>100</sup>

G6PD deficiency may be present in as many as 13% of American Negro males<sup>17,100</sup> and was found in 8.2% of Brazilian Negro males<sup>80</sup> and in 3% of Bantu males.<sup>24</sup> Perhaps 20% of the American Negro population are females heterozygous for G6PD deficiency.<sup>97</sup> In Sardinia, an incidence of 14.35% was recorded in contrast to a very low frequency (0.4%) in the Italian peninsula<sup>82</sup> and 2.7% in Malta.<sup>20</sup> In Northern European populations, the condition is even less common (0.1%). The deficiency has been found in most areas of Greece, but the highest frequencies, 9.5% and even higher, were in the lowland areas.<sup>91</sup> The condition is prevalent among Sephardic and Oriental Jews, and among Kurdish Jews 50% or more may be affected.<sup>100</sup> G6PD deficiency has also been observed in Arabs,<sup>54</sup> Southern Chinese,<sup>21,23,64</sup> and in Filipinos and Thais.<sup>100</sup> It seems to be rare in American Indians.<sup>58,96</sup> The high incidence among certain populations where malaria was once prevalent, as in the Greek lowlands,<sup>91</sup> like thalassemia (Chapter 26), has been attributed to the selective advantage the enzyme deficiency offers against malaria. It has been suggested that the malarial parasites, when harbored in G6PD-deficient red cells, either find that environment inadequate or are more likely to be destroyed in the RES together with their defective host.<sup>5,61</sup> The concept of "balanced polymorphism" is best supported, however, in relation to the geographic inci-

dence of the sickle cell trait and will be discussed in Chapter 25.

### The Enzyme and Its Variants<sup>107</sup>

Normal human G6PD consists of subunits, each having a molecular weight of about 50,000 to 55,000.<sup>51,104</sup> The subunit has pyroglutamic acid at the amino terminal and glycine at the carboxy terminal.<sup>104</sup> Depending on protein concentrations, pH, and the ionic strength of the solution, G6PD exists in various oligomeric forms—tetrameric when associated with NADP or NADPH at neutral pH and high ionic strength, and dimeric at its optimal pH. Functioning G6PD is predominantly dimeric.

That many variants of G6PD have been discovered is not surprising, after what has been learned concerning amino acid substitutions in the hemoglobin molecule (Chapter 24). In contrast to the latter, however, the exact molecular abnormality of most of the variants is unknown. As yet, only the normal enzyme has been purified to a homogeneous, crystalline state and fingerprinted,<sup>101</sup> and only two variants, A<sup>101</sup> and Hektoen,<sup>103</sup> have been shown to be due to single amino acid substitutions.

G6PD may be subjected to electrophoresis on a variety of media and the position of the enzyme can be determined by application of a reaction system that results in precipitation of a dye or the appearance of a fluorescent band at the point to which the enzyme has migrated.<sup>5</sup> The concentration of substrate at which the enzyme manifests one half of its maximum velocity (the Michaelis constant,  $K_m$ , of the enzyme) may be determined, as well as activity at different pH levels, rate of denaturation by heat, and capacity to utilize different substrate analogues. Based on such measurements, standardized methods for characterization of G6PD have been agreed upon internationally,<sup>100</sup> an international reference laboratory has been established, and a standard nomenclature has been recommended.<sup>3</sup>

The genotypic and phenotypic symbol is Gd, that for the enzyme itself is G6PD.<sup>3</sup> The normal enzyme is designated B or B\*, the

plus sign indicating normal enzymatic activity (65 to 150%). This is the most common enzyme found in all population groups studied. Another common variant, A<sup>+</sup>, the one found to be especially prevalent in Negroes, migrates faster than B on electrophoresis. Type A<sup>-</sup> is the most common, clinically significant, abnormal form of G6PD in Negroes. The minus sign indicates enzyme activity of 25% or less. To designate 25 to 65% activity the sign  $\pm$  was proposed, and for greater than 150%, ++, but these signs have only been used in association with the letter designations. Furthermore, they are somewhat misleading in that the enzyme present in the erythrocytes of Gd<sup>A</sup> individuals, although structurally different from the normal, was found to be enzymatically as active as normal G6PD, but fewer active molecules could be detected. In addition, the abnormal enzyme is produced at the same rate as the normal enzyme so that the youngest erythrocytes and

the nucleated red cells of the enzyme-deficient individuals display normal enzyme activity.<sup>101,103</sup> Thus, the greatly reduced activity refers to the red cells of non-anemic individuals, very few of which are young corpuscles. This explains the clinical manifestations of G6PD deficiency in GD<sup>A-</sup> persons (Fig. 23-2).

B and A are the only letter designations for G6PD variants. All other variants are designated by geographical or trivial names (Table 23-1). The common variant with deficient activity in Caucasians is Gd Mediterranean; a variant found among Chinese is Gd Canton.<sup>63</sup> Other common variants<sup>107</sup> are Gd Markham (New Guinea), Gd Taiwan-Hakka (Hakka-Chinese), Gd Union (Philippines),<sup>106</sup> Gd Campbellpur (Pakistan), Gd Debrousse<sup>34</sup> (Arab), Gd Athens (Greece),<sup>60</sup> and perhaps also Gd Corinth (Greece, Mediterranean, S.E. Asia), and Gd Panay (Philippines).<sup>37</sup>

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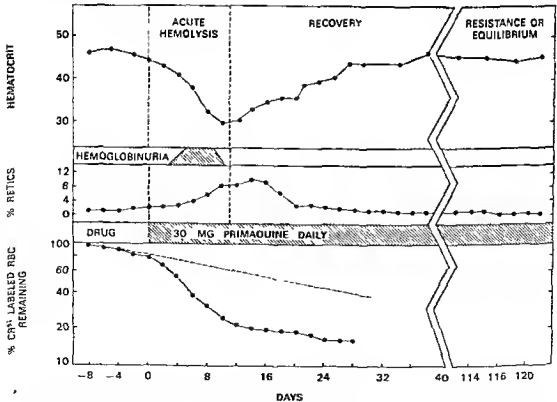


Fig 23-2 The clinical course of primaquine hemolysis based on observations in three G6PD-deficient Negro males (From Alving et al<sup>1</sup> courtesy of the authors and Bulletin of the World Health Organization )

Table 23-1. G6PD Variants

*Severe deficiency associated with congenital, nonspherocytic hemolytic anemia ("Class 1")*

<i>Electrophoretically fast</i>	<i>normal</i>	<i>slow</i>
"Beaujon," Charleston <sup>11</sup>	Albuquerque, <sup>10</sup> Athens like <sup>44a, 72</sup>	Alhambra, <sup>7</sup> Ashdod, Carswell, <sup>54</sup>
Ohio, San Diego, <sup>41</sup>	Bagdad, <sup>37a</sup> Bangkok, <sup>75</sup> Bat-Yam, <sup>75</sup>	Freiburg, Johannesburg, <sup>1a</sup> Mil-
Torrance <sup>74</sup>	Chicago, <sup>10</sup> Clichy, <sup>47</sup> Den Haag, <sup>76</sup>	waukee, Ramat-Gan, <sup>75</sup> Rotter-
	Duarte, <sup>30</sup> Englewood, <sup>76</sup> Hong	dam, <sup>74</sup> Tripler, <sup>34</sup> Worcester <sup>55</sup>
	Kong, Mediterranean, New	
	York, <sup>74</sup> Oklahoma <sup>53</sup> "Paris" (?),	
	Schwaben, Strasbourg	

*Severe enzyme deficiency, usually without non-spherocytic congenital hemolytic anemia ("Class 2")*

<i>Electrophoretically fast</i>	<i>normal</i>	<i>slow</i>
Dakar, <sup>46</sup> Huahien, Huahien	B-Chinese <sup>23</sup> Campbellpur,	Carswell, Lifta, Orchomenos, <sup>72</sup>
Chi, Lublin, Markham, Tai-	Corinth, Indonesia, Mali <sup>46</sup>	Panay, <sup>37</sup> Port Elizabeth <sup>1b</sup>
wan-Ami 1 and 2, Taiwan-	Mediterranean	
Hakka, Teheran, Union, <sup>104</sup>		
Zahringer		

*Moderate to mild enzyme deficiency ("Class 3")*

<i>Electrophoretically fast</i>	<i>normal</i>	<i>slow</i>
A-, <sup>105</sup> Attica, Barbiere,	Columbus, El Morro <sup>44a</sup>	Athens, <sup>70</sup> Benevento, Cape-
Canton, <sup>61</sup> Chibuto <sup>78</sup>		town, Kerala, Mexico, <sup>58</sup>
Debrousse <sup>54</sup> (formerly		Port Royal, Seattle, Tel
Constantine) Kabyle		Hashomer, Washington, West
Melissa, Puerto Rico, <sup>44a</sup>		Bengal
San Juan, <sup>44a</sup> Taipei-		
Hakka, Toronto <sup>72a</sup>		

*Very mild or no enzyme deficiency ("Class 4")*

<i>Electrophoretically fast</i>	<i>normal</i>	<i>slow</i>
A* Inhambane <sup>78</sup> King	B*	Baltimore-Austin, <sup>40</sup> Ibadan
County, Levadia, Lourenzo		Austin, <sup>40</sup> Ijebu-Ode, Ita-Bale,
Marques, <sup>78</sup> Steilacoom		Karditsa, Madrona <sup>40</sup>
		Manjaceze, <sup>78</sup> Minas Gerais,
		Tacoma, Thessaly, Western

*Increased enzyme activity ("Class 5")*

<i>Electrophoretically fast</i>
Hektoen <sup>11</sup>

The variants are listed in five classes according to degree of enzyme activity and the associated clinical manifestations. In addition, each is subdivided according to electrophoretic mobility and under that category, the variants are listed alphabetically and the most common ones are shown in bold face type. Where no references are given, they will be found in Bull WHO<sup>107</sup> in which the reported values for electrophoretic mobility,  $K_m$ , heat stability, etc., are tabulated. Also noted in the same issue are additional variants for which insufficient data have been provided to warrant their inclusion in the above tabulation. In addition are listed the following variants thought to be identical with one of the variants given in the table: Hong Kong 2, Johnstown, Jobit, Loyola (D-), Nashville 2, New Guinea II, Panay-like, Seattle-like, Singapore, U-M<sup>72</sup>

Organization a table listing the biochemical properties of the above-named common forms together with an additional 66 rare variants, for a total of 78 variants, was published<sup>107</sup> and a number of other reported variants, insufficiently described, or possibly identical to variants named previously, were recorded. Still other variants have since been

reported.<sup>46, 47, 69, 76</sup> About half the reported variants have normal or only slightly reduced enzymatic activity in red cells and are not associated with clinical manifestations. These include B\* and A\*. Another group of variants is associated with severe deficiency of enzymatic activity in the red cells, and may or may not require exogenous agents such as

drugs, infections, or fava beans for hemolysis to occur. These include Gd Markham, Gd Union, Gd Campbellpur, Gd Mediterranean, Gd Corinth, and Gd Panay.<sup>37</sup> In still another group the enzymatic deficiency is moderate or mild. This group includes Gd<sup>A</sup>, in which exposure to an oxidant usually is the only circumstance leading to the occurrence of hemolytic anemia, and also Gd Debrousse, Gd Canton, and Gd Athens. About 20 variants are associated with enzymatic deficiency which is not necessarily more than moderate, but chronic nonspherocytic hemolytic anemia is present nevertheless, even in the absence of exogenous agents. These variants include Gd Manchester, Gd Alhambra,<sup>7</sup> and Gd Tripler.<sup>31</sup> One variant, Gd Hektoen,<sup>31</sup> has increased activity.

Some idea of the frequency of rare as compared with common variants of G6PD is gained from a survey of 735 male African Bantus, among whom an average of 63% G6PD B<sup>+</sup> was found, 17% G6PD A<sup>+</sup>, 19% G6PD A<sup>-</sup>, and 1.2% rare variants, which included four probable new ones (G6PD Inhambane, G6PD Chibuto, G6PD Lourenzo Marques, and G6PD Manjacaze).<sup>78</sup>

In the Negro (Gd<sup>A</sup>), G6PD deficiency is largely confined to the erythrocytes, but in Caucasian mutants the defects involve many other tissues, including leukocytes<sup>14,73</sup> and platelets.<sup>74</sup> In one female patient with G6PD deficiency and hemolytic anemia, fatal sepsis was associated with inadequate bactericidal activity resulting from defective PPP shunt activity.<sup>28</sup>

### Mechanisms of Hemolysis

The critical role that reduced glutathione (GSH) plays in red cell metabolism (Chapter 3) makes it necessary for the red cell to be capable of rapidly regenerating GSH whenever it has been oxidized. When this is possible, the erythrocyte can continuously absorb free radicals or peroxide derived from drugs or other sources without harmful consequences to the cell.<sup>55</sup> Administration of primaquine to Gd<sup>A</sup> subjects was observed to be associated with a decrease in the levels of

GSH in the erythrocytes, followed by acute hemolytic anemia. This occurred because the mutant A<sup>-</sup> enzyme, although enzymatically as active as normal G6PD, is more rapidly degraded during erythrocyte aging than is the normal enzyme.<sup>105</sup>

In Gd Mediterranean individuals, on the other hand, enzyme activity is significantly lower than normal even in the youngest red cells, and degradation during the aging of red cells is accelerated. Since the earliest observations on these two variants were made, many other variants and a variety of clinical syndromes associated with G6PD deficiency have been encountered, as already mentioned, but in many instances the degree of enzyme deficiency has not correlated well with the clinical manifestations. Thus, whereas the hemolytic manifestations in Gd Bat-Yam, Gd Ramat-Gan,<sup>75</sup> and Gd Worcester<sup>84</sup> subjects could be explained by the total absence of G6PD activity, and hemolysis in a Gd Oklahoma variant subject<sup>53</sup> could be attributed to a very low affinity of the variant enzyme for G6P (glucose 6-phosphate, Fig. 23-1), in other variant subjects, such as Gd Tripler,<sup>34</sup> Gd Manchester, and Gd Alhambra,<sup>7</sup> chronic hemolytic anemia was present even though neither very severe G6PD deficiency nor unusually high  $K_m$  values for substrate or coenzyme were present.<sup>104</sup> It is reasonable to conclude that enzyme activity and kinetic characteristics determined *in vitro* under unphysiologic conditions may provide misleading information concerning the physiologic activity of the enzyme in the variant cells. For example, whether or not variant enzymes generate enough NADPH in red cells to maintain an adequate concentration of reduced glutathione may depend on whether the enzyme is inhibited by physiologic concentrations of NADPH and by ATP.<sup>104</sup>

The exact reason why the life span of G6PD-deficient red cells is shortened is not known. Drug-induced hemolysis of such cells generally is accompanied by the formation of Heinz bodies (see Chapters 3 and 20) which become attached to the red cell membrane. The Heinz bodies may be produced as the

result of the formation of hydrogen peroxide through interaction of the chemical agent or its active derivative and oxyhemoglobin.<sup>24</sup> In the absence of adequate amounts of GSH, oxidative denaturation of the hemoglobin occurs and is followed by precipitation of globin. The precipitated globin becomes attached through disulfide bridges to membrane sulfhydryl groups<sup>43,56</sup> and appears as Heinz bodies. G6PD-deficient red corpuscles containing such inclusions encounter difficulties in passage through the spleen and perhaps elsewhere with resulting destruction, which occurs both in the spleen and liver.<sup>98</sup>

*Additional mechanisms, possibly involved* in the pathogenesis of hemolysis associated with infection or favism in G6PD-deficient subjects, and in neonates, will be discussed below.

### Clinical Manifestations

The clinical manifestations of G6PD deficiency may be episodic with complete clinical recovery in the intervening periods, as seen most typically in the Negro subjects exposed to primaquine whose illnesses first drew attention to this disorder; or they may be chronic, but often marked by periods of acceleration; or they may occur under conditions of stress associated with infection or drug administration; or they may appear in the neonatal period or after exposure to the fava bean.

### Drug-Induced Hemolytic Anemia

The typical event in Gd<sup>A-</sup> individuals is a self-limited hemolytic episode from which recovery occurs even if drug exposure is continued (Fig. 23-2). The clinical manifestations are those of an acute hemolytic anemia (Chapter 20) of moderate severity, together with the usual hematologic and pigmentary changes and the appearance of Heinz bodies. Abdominal or back pain may occur; the urine may turn dark, even black with hemoglobinuria; and then signs of erythrocyte regeneration appear, during which the Heinz bodies disappear, presuma-

bly because of sequestration and removal in the spleen. In addition to primaquine and pamaquine, a number of other reducing substances ("oxidant drugs") (Table 23-2), especially sulfonamides, cause hemolysis in G6PD-deficient individuals.

By tagging the red cells with radioactive chromium, it was shown<sup>30</sup> that sensitivity to the hemolytic effect of this drug was due to an intrinsic abnormality of the red corpuscles.

**Table 23-2. Compounds Reported to Have Been Associated with Increased Red Cell Destruction in Individuals with G6PD Deficiency<sup>9,14,97,110</sup>**

Antimalarials	Primaquine, pamaquine, pentamquine, quinocidine, quinacrine (Atabrin), quinine (C)
Sulfonamides	Sulfanilamide, sulfapyridine, salicylazosulfapyridine <sup>97</sup> (Azulfidine), sulfamethoxypyridazine (Kynex), sulfacetamide, N <sub>2</sub> -acetylsulfanilamide, 2-amino-5-sulfamylthiazole, sulfisoxazole (Gantisin)
Sulfones	Thiazolsulfone (Promizole), diaminodiphenylsulfone (DDS), sulfoxone (Dapsone) <sup>95</sup>
Nitrofurans	Nitrofurantoin (Furadantin), <sup>50</sup> furazolidone (Furoxone), furaltidone (Altafur), nitrofurazone (Furadin)
Antipyretics and analgesics	Acetanilid, aspirin, acetophenetidin (Phenacetin), antipyrine (C), aminopyrine (Pyramidon) (C)
Miscellaneous	Naphthalene (mothballs), <sup>29</sup> probenecid (Benemid), vitamin K (water soluble analogues) <sup>93</sup> , phenylhydrazine, isoniazid with or without para-aminosalicylic acid, trinitrotoluene, methylene blue, dimer-capitol (BAL), chloramphenicol (C), quinine (C), fava beans (C)

(C) denotes drugs that cause hemolysis in G6PD-deficient Caucasians but are not or are only slightly hemolytic in Gd<sup>A-</sup> Negroes

The sensitivity was noted to be self-limited for, after the initial hemolytic episode, the hemoglobin level returned to normal even though administration of the drug was continued (Fig. 23-2). Red corpuscles obtained from subjects who had undergone hemolysis and were still receiving the drug were no longer sensitive, as judged by the survival of the red cells following transfusion into a patient who had never before received primaquine. Nevertheless, when red cells from a primaquine-sensitive subject, withdrawn long after a hemolytic reaction, were transfused into a subject who had been pretreated with primaquine, the corpuscles were rapidly destroyed. Thus it became clear that, in primaquine-sensitive subjects, some red cells are sensitive and others are not. The nonsensitive ones predominate after a hemolytic reaction. Cohort labeling with  $^{59}\text{Fe}$  revealed that sensitivity was a function of red cell age and this in turn focused attention on the metabolism of the red cells, since it was well known that the activities of certain enzymes decline on aging. The discovery that the level of GSH in primaquine-sensitive cells is diminished and that the cells are unable to protect the GSH against oxidative stress<sup>8</sup> led to examination of the pathways of GSH reduction within the red cells of primaquine-sensitive individuals and the discovery that there was a deficiency of G6PD.<sup>19</sup>

In Gd Mediterranean individuals, whose enzyme deficiency is more severe than that of Gd<sup>A</sup> carriers, some drugs (Table 23-2) that rarely are harmful to Gd<sup>A</sup> carriers may cause red cell destruction, or may do so when given in smaller doses.<sup>68</sup> Furthermore, the hemolytic anemia may not clear spontaneously while drug therapy is continued, because the G6PD content of even the youngest cells is low in the Mediterranean variety. Individuals differ in the intensity of the clinical manifestations. Enzyme levels in heterozygous females have been found to range from very low to normal levels,<sup>97</sup> as might be expected on the basis of the X-inactivation hypothesis, so that sex does not exclude the possibility of a drug-induced hemolytic reaction due to G6PD deficiency.<sup>17</sup>

### *Episodic Hemolytic Anemia in the Absence of Drug Administration*

While certain drugs used in the treatment of infections may aggravate red cell destruction in patients with G6PD deficiency,<sup>62</sup> the opposite may also be true if the treatment relieves the infection.<sup>22,39</sup> What is especially noteworthy is the fact that, in the absence of drug administration, hemolytic episodes, in some cases quite severe<sup>81</sup> and even resulting in acute renal failure,<sup>96</sup> have been observed in G6PD-deficient individuals when they had bacterial or viral<sup>99</sup> infections, diabetic ketoacidosis,<sup>17</sup> acute<sup>26</sup> or chronic hepatitis,<sup>71,81</sup> or nephritis.<sup>17</sup> Such hemolytic episodes have been observed in Caucasians<sup>17,22,42</sup> as well as in Negroes, and in females as well as in males.<sup>17</sup> They have been attributed to the oxidative activity generated by naturally occurring substances, such as ascorbic acid, cysteine, and pyruvic acid,<sup>93</sup> to toxic products formed in the course of disease, or to hydrogen peroxide production by phagocytosing leukocytes.<sup>5</sup> The interesting observation has been made that hemolysis of enzyme-deficient erythrocytes was increased when these cells were exposed *in vitro* to influenza virus, as compared with normal cells.<sup>67</sup>

### *Favism<sup>49,60</sup>*

That exposure to the fava bean is toxic and can even be fatal to some individuals has been known, allegedly, since the time of Pythagoras. The onset of hemolysis is quite sudden. According to a study of 506 patients,<sup>49</sup> it may occur within a few hours following inhalation of the plant's pollen and within one or two days after ingestion of either fresh or dry fava beans. The majority of the 506 subjects were children two to five years of age; the male/female ratio was 6.2/1. The syndrome was severe, even in females, and hemoglobin levels as low as 4 g/dl, and even 1.8 g/dl were encountered.

It is clear that favism affects only individuals with G6PD deficiency<sup>94</sup> and especially those with the Mediterranean phenotype, but an additional factor seems to be required

since not all G6PD-deficient persons,<sup>45,88</sup> even sometimes in the same family, are susceptible. Immunologic factors,<sup>60</sup> pyrimidine derivatives in fava beans capable of oxidizing GSH,<sup>77</sup> an erythrocyte acid phosphatase phenotype,<sup>15</sup> and genetic diversity of the Mediterranean G6PD phenotype<sup>92</sup> have been suggested as causes of the susceptibility. Favism has not been reported in subjects with the A<sup>-</sup> type of G6PD deficiency. There is one report of favism from West China.<sup>33</sup>

### Neonatal Jaundice

Neonatal hyperbilirubinemia without evidence of immunologic incompatibility and unrelated to drugs given to mother or infant is relatively common in G6PD-deficient newborns in Greece,<sup>32</sup> Thailand,<sup>72</sup> and in infants with all genotypes of G6PD mutation in China<sup>67</sup> and elsewhere,<sup>37a</sup> but is rare in newborn Negroes.<sup>59</sup> The jaundice may be severe enough to require exchange transfusion. The susceptibility of G6PD-deficient infants to hemolysis has been attributed to their low blood sugar, "enzymatic immaturity," their low levels of red cell GSH peroxidase (page 789), and perhaps also to drugs, such as vitamin K, given when hepatic drug detoxifying systems are incompletely developed.

### Nonspherocytic Congenital Hemolytic Anemia

Some patients with Gd Mediterranean have chronic hemolytic anemia from infancy or childhood, although most develop hemolysis only under conditions of stress.<sup>10</sup> More usually this syndrome, which clinically is similar to that of nonspherocytic hemolytic anemia due to other causes (Chapter 20), is associated with rare enzyme variants, usually with very low activity or marked instability, such as those listed in Table 23-1, "Class I." In the reported subjects, osmotic fragility was normal and splenectomy was not beneficial. Most of the cases reported have been in Caucasians, but the Gd Charleston<sup>11</sup> and Gd San Diego<sup>41</sup> families were Negroes, as was a family with what might have been the Oklahoma variant.<sup>38</sup>

### Diagnosis and Differential Diagnosis

The clinical importance of G6PD deficiency is probably much greater than is generally appreciated. Furthermore, it is not generally recognized that infections can precipitate mild, and sometimes very severe,<sup>69</sup> hemolytic episodes and that small doses of certain drugs (Table 23-2) may do so in G6PD-deficient patients who have diabetic acidosis, pneumococcal pneumonia, hepatitis, streptococcal pharyngitis, chronic renal disease, malaria or other illnesses.<sup>97</sup> The degree of blood destruction differs considerably from one patient to another, and mild episodes of anemia should not be overlooked. In addition, if the hemolysis has been associated with an infection, reticulocytosis may not be as great as might otherwise be expected. Unless the diagnosis is obvious, the occurrence of a hemolytic episode is a reason for an appropriate test for G6PD deficiency to be performed, among other diagnostic steps (Chapter 20). The caution should be added that a blood enzyme assay, performed after a hemolytic episode, often will not detect G6PD deficiency even if present because the most deficient cells have been destroyed. Such patients need to be tested two to four months after the hemolytic episode.

The hematologic manifestations depend on the severity of the hemolytic episode and may range from slight polychromatophilia and little else, to spherocytosis and signs of red cell fragmentation. In the autohemolysis test (Chapter 20), a moderate increase in hemolysis above normal is found; this is only partially prevented by the addition of glucose or ATP. Heinz bodies should be demonstrable (page 736), but their presence is not specific evidence of G6PD deficiency. The possibility of unstable hemoglobin disease can be ruled out by heat stability or isopropanol stability tests (Chapter 24) and hemoglobin electrophoresis (Chapter 20).

Both screening procedures<sup>100</sup> and quantitative assays for G6PD have been described. The former<sup>5,66</sup> depend on either (1) the reduction of a dye (brilliant cresyl blue, methylene blue, etc), which can be observed visu-



ally; (2) the reduction of methylene blue linked to the reduction of methemoglobin (MHb reduction test)<sup>16,100</sup>; (3) the ascorbate-cyanide test, in which the browning of hemoglobin is observed when it is not protected from peroxidation; or (4) the spot test, involving the visual observation of fluorescence of NADPH formed in the G6PD reaction.<sup>4,6</sup>

In the *spot test*, blood is added to a reaction mixture containing saponin, buffer, glucose-6-PO<sub>4</sub>, and NADP. The mixture is spotted on filter paper after 5 to 10 minutes and is inspected under an ultraviolet hand lamp. This commonly used test,<sup>35</sup> a dye reduction procedure, is a fairly specific one and can be carried out on stored blood.

The *ascorbate-cyanide test*<sup>44</sup> utilizes intact red cells rather than hemolysates and for that reason is superior to other screening tests for the detection of the heterozygous state for G6PD deficiency. A tetrazolium-linked procedure<sup>36</sup> also is a practical and sensitive method for this purpose.

G6PD activity of cells can be estimated quantitatively<sup>68</sup> by adding a carefully measured amount of hemolysate prepared from washed, lysed red blood cells to an assay mixture containing buffer, glucose-6-PO<sub>4</sub>, and NADP, and measuring the rate of NADPH generation, as outlined by a WHO scientific group.<sup>100</sup> A cytochemical method also has been devised whereby, through the use of the tetrazolium salt Nitro-BT (NBT), a semiquantitative estimate of G6PD activity in single cells can be made.<sup>79</sup>

The detection of all female heterozygotes is not possible, but those with rather low enzyme levels who are likely to develop hemolytic episodes can be identified by the GSH reduction-inhibition test<sup>108</sup> or the MHb reduction test.<sup>16</sup>

## Treatment

The principles governing the treatment of acute and chronic hemolytic anemias are as applicable here as in hemolytic anemias due to other causes (Chapter 20). G6PD individuals should avoid drugs that might induce

hemolytic episodes (Table 23-2). Whether blood transfusion should be given depends on the rate of hemolysis. If the rate is very severe, as in some cases of favism, whole blood or packed red cells may be lifesaving. Splenectomy has not proved helpful in hemolytic anemia due to G6PD deficiency.

## Course and Prognosis

Unlike the hemolytic episodes in the A<sup>-</sup> type of G6PD deficiency, those occurring in the more severe Mediterranean type of deficiency may not terminate rapidly, but in the vast majority recovery does take place. When the course of the hemolytic process is prolonged, complications such as gallstones may develop, but other complications are unusual. There is little information regarding the ultimate prognosis of people affected with G6PD deficiency.<sup>70</sup>

Favism, however, stands apart. Without adequate treatment, death during a hemolytic episode may occur; fatalities were not uncommon at one time.

## Importance of Screening for G6PD Deficiency

Hemolytic anemia due to G6PD deficiency, as described above, can be severe and even life-threatening. Consequently, screening should be mandatory for all patients of certain ethnic groups on admission to a hospital since they may have illnesses or receive drugs that would produce hemolytic crises.<sup>66</sup> The Mediterranean variant is particularly sensitive. Screening also is desirable for populations of appropriate ethnic background who receive antimalarial prophylaxis. Although standard prophylaxis produces only mild hemolysis in G6PD-deficient Negroes, about 2% of those in the US Army develop severe hemolysis.<sup>66</sup>

It should also be noted that the use of G6PD-deficient blood for transfusion is potentially harmful since patients given such blood may receive drugs or suffer from illnesses that may lead to hemolysis of G6PD-deficient cells. Especially inadvisable is the

use of red cells from donors with the Mediterranean type of G6PD deficiency because a large proportion of such cells may be destroyed.

## Other Deficiencies Involving the HMP (PP) Pathway and GSH Metabolism

In addition to G6PD, deficiencies of four other enzymes involved in the HMP pathway or in GSH metabolism have been described.

### 6-Phosphogluconic Dehydrogenase (6PGD) Deficiency

It is not certain that the reported cases<sup>124</sup> of 6PGD deficiency (Fig. 23-1) represent hemolytic anemia due to this deficiency. In any event, the deficiency must be very rare and the clinical effects are poorly defined. Both partial<sup>116,138</sup> and complete<sup>137</sup> deficiency have been reported. Inheritance is autosomal and diagnosis is made by carrying out an assay for the enzyme.<sup>5</sup>

### Glutathione Reductase (GR) Deficiency

As discussed in Chapter 3 (page 103), because GR is a flavoprotein its activity depends in part on the dietary intake of riboflavin. Consequently, partial deficiency of red cell GR is relatively common. Whether GR deficiency was the cause of the many disorders with which it was found to be associated<sup>126,142</sup> is uncertain. However, restudy of the propositus of one large family following the addition of flavine adenine dinucleotide (FAD) suggested that a true, genetically determined deficiency of GR may exist.<sup>111</sup> Nevertheless it remains very doubtful whether drug-induced or spontaneous pancytopenias<sup>131</sup> or other hemolytic anemias<sup>121</sup> and a variety of reported hematologic disorders are etiologically related to GR deficiency.<sup>5</sup> Although a fluorescent spot screening test<sup>4</sup> will provisionally indicate decreased erythrocyte GR deficiency, definitive diagnosis depends upon specific enzyme assay.<sup>5</sup>

A GR variant was reported to be associated with gout.<sup>127</sup>

### Glutathione Peroxidase (GSH-Px) Deficiency

Erythrocytes from the healthy newborn infant have from 55<sup>117</sup> to 85%<sup>143</sup> of the mean GSH-Px activity found in the erythrocytes of adults.<sup>120,132</sup> Values for premature infants do not differ from those for term infants.<sup>117,120,143</sup> In some studies, adult levels were found to be achieved at 6 to 10 months,<sup>132,143</sup> but, in another series,<sup>117</sup> not until puberty. As contrasted with the erythrocytes of adults, erythrocytes of normal newborn infants form Heinz bodies and methemoglobin more readily upon exposure to low concentrations of hydrogen peroxide *in vitro*.<sup>120</sup> This has been attributed to their relative deficiency of GSH-Px, and has been correlated with a very mild, self-limited hemolytic process found in four newborn infants with a partial deficiency of GSH-Px.<sup>132</sup> Neonatal jaundice was reported to be associated with partial deficiency of GSH-Px in 12 infants.<sup>134</sup> In a large prospective study, however, relatively low levels of GSH-Px in newborn infants did not correlate with greater degrees of jaundice.<sup>143</sup>

In some of the families of the infants with neonatal jaundice, siblings or parents were found to have about 64% of normal adult GSH-Px activity.<sup>134</sup> This observation and the discovery of 70% of normal activity in the parents of a 17 year old boy who was found to have 36% of normal GSH-Px activity and had developed an acute hemolytic anemia associated with Heinz bodies in circulating erythrocytes following the transfusion of 3 units of autologous, stored blood<sup>133</sup> led to the postulate that GSH-Px deficiency is transmitted as an autosomal recessive.<sup>133,134</sup>

Circumstantial evidence favors some role for GSH-Px deficiency in the pathogenesis of transient hemolytic disorders, perhaps in relationship to drugs,<sup>134</sup> vitamin E deficiency in the newborn,<sup>117</sup> or other factors.<sup>143</sup> Menadione bisulfite, the vitamin K analogue,

which may be given to mothers during labor or to the newborn, inactivates GSH-Px.<sup>111</sup> In two adults, partial deficiency of GSH-Px was associated with an anemia which occurred following the administration of sulfonamides and had a hemolytic component.<sup>113</sup> However, in view of reports of deficiency of GSH-Px in apparently unrelated conditions (in the erythrocytes of iron-deficiency anemia,<sup>139</sup> and of liver disease with acanthocytosis,<sup>119</sup> as well as in the platelets of three unrelated patients with Glanzmann's thrombasthenia<sup>122</sup>) one must be cautious about assigning in all the reported cases a pathogenetic relationship between GSH-Px activity and hemolytic disorders.

Various methods for the measurement of GSH-Px have been described<sup>120,129,131</sup> but most consider the assay method of Paglia and Valentine<sup>130</sup> to be the best.

### Glutathione Deficiency

First discovered in Holland,<sup>135</sup> GSH deficiency has since been reported from France,<sup>112</sup> Switzerland,<sup>125</sup> and the United States.<sup>130</sup> The defect may be transmitted as an autosomal recessive trait,<sup>130</sup> but dominant-autosomal inheritance also has been reported.<sup>125</sup> As described in Chapter 3 (page 104), GSH synthesis takes place in two steps. In two cases the defect was found to involve the second step, GSH synthetase,<sup>112,130</sup> but in another the defect resided in the first step, namely,  $\gamma$ -glutamyl-cysteine synthetase.<sup>125</sup> The clinical manifestations are those of a nonspherocytic congenital hemolytic anemia and accentuation of hemolysis may take place during drug administration. In the patient with GSH synthetase deficiency, both the Heinz body test and the cyanide-ascorbate test gave positive reactions.<sup>130</sup> GSH deficiency is diagnosed by measuring the content of the tripeptide in red cells,<sup>110</sup> but localization of the enzyme defect in one of the two steps of GSH synthesis requires specific enzyme assays which are not carried out in clinical laboratories.<sup>112,123</sup>

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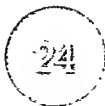
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# *The Hemoglobinopathies: Structural Abnormalities in Globin. General Principles. Unstable Hemoglobin Disease*

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## History

The story of the growth of knowledge of the hemoglobinopathies and thalassemia pro-

vides a fascinating picture illustrating the value of the pursuit of knowledge for its own sake. It reveals the fruits that can be gained if curiosity is aroused and an answer sought to questions that may at the time seem to be of no practical importance, as well as the progress that can be made when the right question is put to a "prepared mind."

In 1910, James B. Herrick<sup>2</sup> reported "peculiar, elongated, sickle-shaped red corpuscles" in "a case of severe anemia" in a Negro. He described the clinical manifestations in his patient in considerable detail. The sickle cells, he thought, were freakish poikilocytes, and, with considerable prescience, suggested that they were a manifestation of a peculiar chemical or physical condition. Emmel, in 1917, observed the transformation of the biconcave disc to the sickle form *in vitro*.<sup>3</sup> He also noted that sickling occurred both in persons with severe anemia and in others who were apparently healthy, thus recognizing both sickle cell anemia and sickle cell trait.

The comprehensive studies of Hahn and Gillespie in 1927<sup>4</sup> delineated the conditions affecting sickle cell formation *in vitro*, including pH, temperature, fixatives, tonicity and others. Among the most important of

their observations was that exclusion of oxygen was a prerequisite to sickling and that the phenomenon could be reversed on reexposure to the gas. They postulated that similar effects of oxygen could occur in vivo, hypoxia leading to cellular distortion with consequent hemolysis. Later, Hahn applied the term "sickle cell trait" to the asymptomatic condition associated with in vitro sickling. He performed family studies and concluded that the trait was inherited as a dominant character.

Paralleling these events was the report of Cooley and Lee, in 1925, who separated from the complex of disorders of infancy and childhood that had been known as von Jaksch's anemia a syndrome characterized by chronic, progressive anemia commencing early in life, with pronounced erythroblastosis in the blood, a characteristic facies, splenomegaly, and a familial incidence.<sup>2</sup> The observation that these patients were of Mediterranean background led to the introduction of the name "thalassemia," derived from the Greek word for sea. The parents of these children, unfortunately, were not examined. At this time and in the following years, descriptions appeared in the Italian literature of a milder disorder, encountered in adults as well as children, which was marked by morphologic abnormalities in the red cells and evidence of increased hemolysis, in spite of decreased red cell osmotic fragility (microcytopenic anemia, *malattia di Rietti-Greppi-Micheli*). In 1938, Caminopetros noted that the parents of a child suffering from severe thalassemia had diminished red cell osmotic fragility,<sup>3</sup> but even then the true relationship between Cooley's anemia and the Rietti-Greppi-Micheli syndrome was not appreciated.

Curiosity regarding the significance of basophilic stippling of the red cells of several adult patients with minor ailments led to the description in 1940 by Wintrobe and his associates of what they considered to be a mild form of Cooley's anemia.<sup>12</sup> These investigators also showed that the manifestations of this disorder were present in both parents of a child with classic Cooley's

anemia. Subsequent formal genetic studies established that Cooley's anemia is the homozygous state for a partially dominant autosomal gene, the patients described by Rietti and by Wintrobe and their co-workers representing the heterozygous state.<sup>10</sup>

In 1940, the sickling phenomenon was re-investigated by Sherman,<sup>9</sup> who confirmed the observations of Hahn and Gillespie regarding reversibility and the importance of oxygen. Sherman also found that the cells in sickle cell disease were birefringent, an observation that aroused curiosity but remained unexplained for nearly a decade. Then, in a casual conversation, the birefringence was called to the attention of the physical chemist, Linus Pauling.<sup>11</sup> Pauling conceived the possibility that interaction between abnormal hemoglobin molecules might explain this phenomenon. With Itano, he demonstrated an electrophoretically abnormal hemoglobin in sickle cell anemia,<sup>8</sup> thus originating the concept of molecular disease.

In the same year that Pauling made his discovery, Neel published a report of elegant genetic studies establishing that sickle cell trait was the heterozygous and sickle cell anemia the homozygous state for the same gene.<sup>7</sup> With the demonstration by Ingram of a difference in the amino acid sequence in one small part of the polypeptide chains of sickle cell hemoglobin,<sup>6</sup> the science of molecular biology took root. The contributions of Perutz, Lehmann, and many others have resulted in an expansion of knowledge to a degree unforeseen even in the 1950's, let alone when Herrick wrote about his patient with a peculiar anemia.

### Definitions

Inherited abnormalities of hemoglobin synthesis may be divided into two groups: those associated with structurally abnormal hemoglobin variants and those in which one or more of the normal hemoglobin polypeptide chains are synthesized at a markedly reduced rate. The term "hemoglobinopathy" will be restricted to the former disorders and the term "thalassemia" to the latter. Included



with the thalassemias are certain disorders in which structurally abnormal hemoglobins are formed from normal chains or parts of normal chains, such as Hb H ( $\beta_4$ ), Hb Bart's ( $\gamma_4$ ), and Hb Lepore. Hemoglobin Constant Spring also is included with the thalassemias because of the clinical picture it produces (page 881).

## Etiology and Pathogenesis

### Structure of the Abnormal Hemoglobins

The structure of normal hemoglobin is discussed in Chapter 4 (page 172) and the amino acid sequence of the normal  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -chains is given in Table 4-7 (page 174).

The great majority of abnormal hemoglobins differ from the normal in that a single amino acid has been substituted for another. In hemoglobin S, for example, valine has replaced glutamic acid as the sixth amino acid from the N-terminal end of the  $\beta$ -chain. Many other examples of this kind of substitution have been described. These are listed in Table 24-1 according to whether the mutation has affected the  $\alpha$ -chain, the  $\beta$ -chain, the  $\gamma$ -chain, or the  $\delta$ -chain. Many more  $\beta$ -chain variants than  $\alpha$ -chain variants have been reported, possibly because serious abnormalities of the  $\alpha$ -chain might be expected to cause early fetal death, thereby making their discovery less likely. Since fetal hemoglobin ( $\alpha_2\gamma_2$ ) does not contain  $\beta$ -chains, an abnormal  $\beta$ -chain is unlikely to produce fetal death and therefore has a greater chance to persist until its subject has reached an age at which the disorder will be detected. The paucity of reported  $\gamma$ - and  $\delta$ -variants presumably reflects the fact that structural alterations in these chains are not apt to produce a disease state that would bring them to the attention of a physician.

In a small group of hemoglobins, the abnormalities are more complex than amino acid substitution (Table 24-2). Two amino acid substitutions are present in HbC Harlem, HbC Georgetown, and HbJ Singapore,

and three in Hb Hopkins 2-II.<sup>106</sup> Extra amino acids have been added to the C-terminal ends of hemoglobins Constant Spring<sup>86</sup> and Tak,<sup>90</sup> whereas Hb Wayne not only has additional amino acids, but also has an altered sequence affecting the C-terminal residues.<sup>117</sup> The Lepore hemoglobins (Chapter 26) are those in which the non- $\alpha$ -chain is a hybrid formed from the N-terminal portion of the  $\delta$ -chain and the C-terminal portion of the  $\beta$ -chain. "Anti-Lepore" hemoglobins (Chapter 26), ie, with a  $\beta$ -N-terminal and a  $\delta$ -C-terminal, have also been described; eg, Hb Miyada and HbP Congo.<sup>47</sup> A possible  $\gamma\beta$  hybrid, Hb Kenya, has been reported.<sup>95</sup> Deletions of one or more amino acids are found in several hemoglobins (Table 24-2). Finally, some hemoglobins are tetramers, ie, all four polypeptide chains are identical. The genetic mechanisms leading to these complex changes are discussed in a later section (page 797).

### Nomenclature

When newly discovered hemoglobins were first reported, they were designated by letters of the alphabet. Normal adult hemoglobin and fetal hemoglobin were called HbA and HbF, respectively. When sickle cell hemoglobin was discovered, some called it HbB, but soon the letter S was assigned to it, and, to avoid confusion, no other hemoglobin was designated B.<sup>36</sup> Hemoglobins associated with methemoglobinemia were given the letter M.<sup>55</sup> Other hemoglobins were assigned letters in alphabetical order. By the time the letter Q was reached, it had become apparent that this system would not provide enough designations. Furthermore, structurally different hemoglobins occasionally had been given the same letter. In 1960 a new system of nomenclature was adopted, and the letters R and T through Z were left unassigned.<sup>38</sup>

New hemoglobins are now given geographic names, and it is left to the discoverer to choose the most appropriate one. The name may be a city, district, province, hospital, etc, and may be the native region of the propositus or the place of discovery. Capital

letters are sometimes retained to indicate the electrophoretic mobility most nearly approximated by the hemoglobin variant. Thus, the designation "HbG Copenhagen" indicates a hemoglobin discovered in Copenhagen and found to have the same electrophoretic mobility as that of other hemoglobins G.

The nomenclature also provides for an abbreviated designation that indicates the nature of the structural abnormality insofar as it is known. Hemoglobin A is represented as  $\alpha_2\beta_2^A$  (see Chapter 4, page 172). When the abnormal chain has been determined, the name is substituted for the superscript of the abnormal chain, as  $\alpha_2\beta_2^{Punjab}$ . When the abnormality can be localized to a particular tryptic peptide (page 810), the designation becomes  $\alpha_2\beta_2^{T_{p1}}$ , indicating that the first tryptic peptide of the  $\beta$ -chain is defective. When the amino acid substitution in the abnormal peptide is known, it is indicated; eg,  $\alpha_2\beta_2^{T_{p1} \text{ Glu} \rightarrow \text{Val}}$ . Finally, when the nature and location of the substitution have been defined precisely, the abbreviation indicates the position (sequential number) and name of the substituted amino acid. Thus, HbS is designated  $\alpha_2\beta_2^{S_{65} \text{ Val}}$  or  $\alpha_2\beta_2^{S_{65} \text{ Glu} \rightarrow \text{Val}}$ . Many investigators now also include in parentheses the helical designation (Chapter 4) for the site of amino acid substitution, in which case HbS might be indicated by the formula  $\alpha_2\beta_2^{S_{65}(\text{A3}) \text{ Glu} \rightarrow \text{Val}}$ .

## Genetics

### Allelism and Linkage

By means of hybridization of radioactive mRNA with human chromosomes in metaphase, it was demonstrated that there are two chromosomal loci involved in hemoglobin synthesis, one on chromosome 2 and another on a B group chromosome (number 4 or 5)<sup>110</sup> (see Fig. 2-3, page 45). From the size of the two loci, it was concluded that the  $\alpha$ -chain structural genes occupy the locus on chromosome 2, whereas the  $\beta$ -,  $\gamma$ -, and  $\delta$ -structural genes occupy the B group locus.

That the  $\alpha$ -locus includes two separate, non-allelic, possibly reduplicated, structural

genes was suggested<sup>101</sup> to explain the generally lower proportion of abnormal hemoglobin found in patients with  $\alpha$ -chain variants (see below), the relatively mild nature of  $\alpha$ -thalassemia minor, and certain puzzling aspects of the inheritance of hemoglobin H disease<sup>121</sup> (Chapter 26). This concept has been supported by the detection of three structurally different  $\alpha$ -chains in members of three different families.<sup>84,92,106</sup> However, the absence of normal  $\alpha$ -chains in patients homozygous for Hb J Tongariki is difficult to explain on this basis.<sup>81</sup>

Genetic evidence indicates that the  $\beta$ - and  $\delta$ -genes are closely linked<sup>93,103,112</sup> and such linkage is further supported by the occurrence of asymmetric crossovers (page 874) between these genes, forming hemoglobins of the Lepore and Miyada type (Table 24-2; also Chapter 26). It is also likely that the  $\gamma$ -locus is near the  $\beta\delta$ -locus, since a  $\gamma\beta$ -hybrid, hemoglobin Kenya, has been reported.<sup>95</sup> There is good evidence that there are at least two non-allelic, structural  $\gamma$ -genes, one for a fetal hemoglobin designated  $G_\gamma$  ( $\gamma 136$  [H14] glycine) and another for one called  $A_\gamma$  ( $\gamma 136$  [H14] alanine).<sup>113</sup> These hemoglobins are found in normal cord blood in a  $G_\gamma$  to  $A_\gamma$  ratio of 3:1. Evidence from the observed  $\beta\delta$  and  $\gamma\beta$  crossovers suggests that the chromosomal arrangement of genes is  $G_\gamma A_\gamma \beta \delta$ .<sup>95</sup>

All genes for abnormal  $\beta$ -chain variants are alleles,<sup>105</sup> i.e., genes that occupy the same genetic locus. Furthermore, the genes producing  $\beta$ -thalassemias and hereditary persistence of hemoglobin F are either allelic or very closely linked (pseudoallelic) with those producing the  $\beta$ -chain structural variants.<sup>87,103,115</sup> Similarly, the genes producing  $\alpha$ -chain structural abnormalities and the  $\alpha$ -thalassemias probably are alleles. However,  $\alpha$ - and  $\beta$ -chain abnormalities are not alleles, nor are they linked.<sup>97,119,123</sup>

### Patterns of Inheritance

The abnormal hemoglobin diseases are inherited in a fashion sometimes referred to as "autosomal codominant." If the properties of the hemoglobin are such that symptoms

Table 24-1. Hemoglobin Variants Resulting from Single Amino Acid Substitutions\*

Alpha Chain Variants					
Name	Position & Substitution†	Functional Abnormalities‡	Name	Position & Substitution‡	Functional Abnormalities‡
Aida	64(E13) Asp → Asn		J Sardinia	50(CDB) His → Asp	
Ann Arbor	80(F1) Leu → Arg	U	J Tonganku <sup>197</sup>	115(GH3) Ala → Asp	
Atago	85(F6) Asp → Tyr		J Toronto	5(A3) Ala → Asp	
Belinson	Same as L Ferrara		Kagoshima	Same as Norfolk	
Bibba	136(H19) Leu → Pro	U	Knoxville 1	Same as G Philadelphia	
Broussais	90(FG2) Lys → Asn		L Ferrara	47(CD5) Asp → Gly	U
Buda <sup>42</sup>	61(E10) Lys → Asn		L Persen Gulf	57(E6) Gly → Arg	
Chad	23(B4) Glu → Lys		Lapin	29(B10) Leu → Val	
Chesapeake	92(FG4) Arg → Leu	E	M Boston	68(E7) His → Tyr	M
Chupas	114(GH2) Pro → Arg		M Gothenburg	Same as M Boston	
Columbia	Same as L Ferrara		M Iwate	87(F8) His → Tyr	M
D St Louis	Same as G Philadelphia		M Kankakee	Same as M Iwate	
D Washington	Same as G Philadelphia		M Osaka	Same as M Boston	
Dakar	112(G19) His → Gln	u	Mahdof	Same as O (SE Asia)	
Danmark Hill <sup>44</sup>	95(G2) Pro → Ala	e	Manitoba	102(G9) Ser → Arg	
Etobicoke	84(F5) Ser → Arg	u	Memphis	23(B4) Glu → Gln	
Fort Worth <sup>34</sup>	27(B8) Glu → Gly		Mexico	54(E3) Gln → Glu	
G Audhali	23(B4) Glu → Val		Nishkur	Same as Norfolk	
G Azuokoki	Same as G Philadelphia		Norfolk	57(E6) Gly → Asp	
G Bristol	Same as G Philadelphia		O Indonesia	118(GH4) Glu → Lys	
G Chinese	30(B11) Glu → Gln		O Padua	30(B11) Glu → Lys	
G Georgia	95(G2) Pro → Leu	ua	O India <sup>39</sup>	64(E13) Asp → His	
G Hong Kong	Same as G Chinese		O Iran	75(EF4) Asp → His	
G Honolulu	Same as G Chinese		O Thailand	74(EF3) Asp → His	
G Norfolk	85(F6) Asp → Asn		Rampa <sup>35</sup>	95(G2) Pro → Ser	ue
G Philadelphia	68(E17) Asn → Lys		Russ	51(CDB) Gly → Arg	
G Singapore	Same as G Chinese		Sealy	Same as Hasharon	
G Taichung	Same as O Thailand		Setif <sup>46</sup>	94(G1) Asp → Tyr	
Hasharon <sup>34</sup>	47(CD5) Asp → His	u	Shimonoseki	54(E3) Gln → Arg	
Hikoshima	Same as Shimonoseki		St Lukes <sup>34</sup>	95(G2) Pro → Arg	
Hopkins 2 1 <sup>34</sup>	112(G19) His → Asp	u	Sina	Same as Hasharon	
I	16(A14) Lys → Glu	A	Singapore	141(HC3) Arg → Pro	
I Interlaken	Same as J Oxford		Stanleyville 2	78(EF7) Asn → Lys	
J Abdjan	51(CD8) Gly → Asp		Tagawa 1	Same as Broussais	
J Capetown	92(FG4) Arg → Gln	E	Tagawa-2	Same as L Ferrara	
J Medellin	22(B3) Gly → Asp		Tonno <sup>38</sup>	43(CD1) Phe → Val	Uc
J Oxford	16(A13) Gly → Asp		Ube 2	68(E17) Asn → Asp	
J Paris	12(A10) Ala → Asp		Umi	Same as L Ferrara	
J Paris-2	Same as Mexico		Yukuhashi-2	Same as L Ferrara	
J Rajapen <sup>41</sup>	90(FG2) Lys → Thr		Zambia	60(E9) Lys → Asn	
Beta-Chain Variants					
Abruzzo <sup>40</sup>	143(H21) His → Arg		E	26(B8) Glu → Lys	C
Agenoge	90(F6) Glu → Lys	C	E Saskatoon	22(B4) Glu → Lys	
Bethesda <sup>32</sup>	145(HC2) Tyr → His	E	G Accra	79(EF3) Asp → Asn	
Borås	88(F4) Leu → Arg	Uc	G Copenhagen <sup>208</sup>	47(CD6) Asp → Asn	
Bingham	100(G2) Pro → Leu	E	G Coughatta	22(B4) Glu → Ala	
Bristol <sup>58</sup>	67(E11) Val → Asp	Uc	G Galveston	43(CD2) Glu → Ala	
Bryn Mawr <sup>34</sup>	85(F1) Phe → Ser	Uc	G Hsi-Tsao <sup>31</sup>	79(HC3) Asp → Gly	
Bucuresti <sup>30</sup>	42(CD1) Phe → Leu	Uc	G Makassar <sup>31</sup>	6(A3) Glu → Ala	
C	6(A3) Glu → Lys	A	G Port Arthur	Same as G Galveston	
Casper <sup>235</sup>	106(G8) Leu → Pro	Us	G San Jose	7(A4) Glu → Gly	
Christchurch <sup>33</sup>	71(E15) Phe → Ser	U	G Saskatoon	Same as G Coughatta	
D Bushman	16(A13) Gly → Arg		G Suzhu	80(EF4) Asn → Lys	
D Chicago	Same as D Punjab		G Taiwan-Amu	25(B7) Gly → Arg	
D Cyprus	Same as D Punjab		G Texas	Same as G Galveston	
D Ibadan	87(F3) Thr → Lys		Genova <sup>33</sup>	28(B10) Leu → Pro	U
D Iran	22(B4) Glu → Gln		Gifu <sup>39</sup>	Same as G Suzhu	
D Portugal	Same as D Punjab		Hammersmith <sup>225</sup>	42(CD1) Phe → Ser	UC
D Los Angeles	Same as D Punjab		Hijiyama	120(GH3) Lys → Glu	
D Punjab	121(GH4) Glu → Gln		Hikari	61(E5) Lys → Asn	
Deer Lodge <sup>44</sup>	2(NA2) His → Arg		Hirono	37(C3) Try → Ser	E
Ohofar	58(E2) Pro → Arg		Hiroshima	146(HC3) His → Asp	E

Table 24-1 (Continued)

Beta-Chain Variants (Continued)					
Name	Position & Substitution†	Functional Abnormalities‡	Name	Position & Substitution†	Functional Abnormalities‡
Hiroshima	Same as Hqiyama		N Seattle	61(E5) Lys → Glu	
Hofu	126(H4) Val → Glu		Nagasaki	17(A15) Lys → Glu	
Hope	136(H14) Gly → Asp		New York	113(G15) Val → Glu	
Hopkins-1	Same as N Baltimore		O Arab	121(GH4) Glu → Lys	
I High Wycombe	59(E3) Lys → Glu	U	Oak Ridge <sup>42</sup>	Same as D Punjab	
I Toulouse	66(E10) Lys → Glu	U	Ocha Ries <sup>39</sup>	52(D3) Asp → Ala	
Istanbul <sup>31</sup>	92(F8) His → Glu		Olmsted	141(H19) Leu → Arg	U
J Baltimore	16(A13) Gly → Asp		Olympia <sup>37</sup>	20(B2) Val → Met	E
J Bangkok	56(D7) Gly → Asp		Osaka Christianborg	52(D3) Asp → Asn	
J Cambridge <sup>20a</sup>	69(E13) Gly → Asp		P	117(G19) His → Arg	
J Iran	77(EF1) His → Asp		Perth	32(B14) Leu → Pro	
J Ireland	Same as J Baltimore		Peterborough <sup>45</sup>	111(G12) Val → Phe	Uc
J Kaohsiung <sup>25</sup>	59(E3) Lys → Thr		Philly	35(C1) Tyr → Phe	U
J Korat	Same as J Bangkok		Porto Alegre	9(A6) Ser → Cys	A
J Meining	Same as J Bangkok		Ramier <sup>22</sup>	145(HC2) Tyr → Cys	E
J Taichung	129(H7) Ala → Asp		Richmond	102(G4) Asn → Lys	e
J Thailand	Same as J Bangkok		Riverdale Bronx	24(B6) Gly → Arg	U
J Trinidad	Same as J Baltimore		Rush <sup>21</sup>	101(G3) Glu → Gln	U
Jenkins	Same as N Baltimore		S	6(A3) Glu → Val	A
K Ibadan	46(CD5) Gly → Glu		Sabine	91(F7) Leu → Pro	U
K Woolwich	132(H10) Lys → Gln	Cu	Saint Etienne <sup>34</sup>	92(F8) His → Gln	Ue
Kansas	102(G4) Asn → Thr	E	St Louis <sup>315</sup>	28(B10) Leu → Gln	U
Kempsey	99(G1) Asp → Asn		San Diego <sup>30a</sup>	109 (G11) Val → Met	E
Kenwood	143(H21) His → Asp		Samia Ana	88(F4) Leu → Pro	U
Khartoum	124(H2) Pro → Arg	u	Savannah <sup>40</sup>	24(B6) Gly → Val	U
Koln <sup>22</sup>	98(FG5) Val → Met	Ue	Seattle <sup>24a</sup>	78(E20) Ala → Glu	UC
Korie Su <sup>38</sup>	73(E17) Asp → Asn		Shepherds Bush <sup>43</sup>	74(E18) Gly → Asp	Ue
Liberian 1	Same as N Baltimore		Sirraz	7(A4) Glu → Cys	
Little Rock <sup>21</sup>	143(H21) His → Gln	E	Sogn <sup>30</sup>	14(A11) Leu → Arg	u
Louisville <sup>44</sup>	42(CD1) Phe → Leu	Uc	Southampton <sup>42</sup>	106(G8) Leu → Pro	U
M Akita	Same as M Hyde Park		Sydney <sup>222</sup>	67(E11) Val → Ala	U
M Chicago	Same as M Saskatoon		Tacoma	30(B12) Arg → Ser	u
M Emory	Same as M Saskatoon		Taipei	22(B4) Glu → Gly	
M Hyde Park	92(F8) His → Tyr	Mu	Ta Li <sup>24</sup>	83(EF7) Gly → Cys	A
M Kuruma	Same as M Saskatoon		Tokachi	2(NA2) His → Tyr	
M Milwaukee	67(E11) Val → Glu	M	Ube <sup>130a</sup>	Same as Koln	
M Radom	Same as M Saskatoon		Wien	130(H8) Tyr → Asp	U
M Saskatoon	63(E7) His → Tyr	M	Yakima	99(G1) Asp → His	E
Malmö	97(FG4) His → Gln	E	Yoshitake	108(G10) Asn → Asp	c
N	Same as N Baltimore		Ypsilanti	99(G1) Asp → Tyr	E
N Baltimore	95(FG2) Lys → Glu		Yukhashi	Same as Dofar	
N New Haven	Same as J Baltimore		Zunch <sup>270</sup>	63(E7) His → Arg	Ue

## Gamma-Chain Variants

F Alexandra	12(A9) Thr → Lys	F Malta	117(G19) His → Arg (136Gly)
F Dickenson	97(FG4) His → Arg		
F Hull	121(GH4) Glu → Lys	F Texas I	5(A2) Glu → Lys
F Jamaica	61(E5) Lys → Glu (136Ala)	F Texas II	6(A3) Glu → Lys

## Delta Chain Variants

A <sub>2</sub>	16(A13) Gly → Arg	Flatbush	22(B4) Ala → Glu
B <sub>2</sub>	Same as A <sub>2</sub>	NYU	12(A9) Asn → Lys
Babinga	136(H14) Gly → Asp	Indonesia <sup>47</sup>	69(E13) Gly → Arg
		Sphakia	2(NA2) His → Arg

\*Where no reference is given, structures are taken from Lehmann et al.<sup>44</sup>  
 †Sequential number is given followed by helical number in parentheses. Amino acid abbreviations are explained in Table 4-7, page 174.

‡Functional abnormalities: Aggregation  
 Unstable  
 Increased oxygen affinity  
 Decreased oxygen affinity  
 Methemoglobinemia  
 A severe, a mild: see Chapter 25  
 U severe, u mild: see Table 24-8  
 E with erythrocytosis, a mild: see Chapter 30, Table 30-3  
 C with cyanosis, c mild  
 M, see Chapter 31, Table 31-2

Table 24-2. Hemoglobin Variants Resulting from Multiple Substitutions, Additions, Deletions, and Tetramers

Multiple Substitutions and Additions		
Name	Structural Abnormalities	Functional Abnormalities*
C Georgetown	$\beta 6$ (A3) Glu $\rightarrow$ Val another unidentified	A
Constant Spring <sup>46</sup>	31 extra amino acids at $\alpha$ C-terminal end	
C Harlem	$\beta 6$ (A3) Glu $\rightarrow$ Val 73 (E17) Asp $\rightarrow$ Asn	A
Hopkins 2 II <sup>106</sup>	$\alpha$ 112 (G19) His $\rightarrow$ Asp 114 (GH2) Pro $\rightarrow$ Ser, 118 (H1) Thr $\rightarrow$ Gly	
J Singapore <sup>28</sup>	$\alpha$ 78 (EF7) Asn $\rightarrow$ Asp 79 (EF8) Ala $\rightarrow$ Gly	
Kenya <sup>95</sup>	$\gamma$ - $\beta$ hybrid	
Lepore (several)	$\delta$ - $\beta$ hybrid	
Miyada <sup>51</sup>	$\beta$ - $\delta$ hybrid	
P Congo <sup>42</sup>	$\beta$ - $\delta$ hybrid	
Tak <sup>92</sup>	8 extra amino acids at $\beta$ C-terminal end	
Wayne <sup>117</sup>	$\alpha$ 139 (HC1) Lys $\rightarrow$ Asn 141 (HC3) Arg $\rightarrow$ Val and 5 extra amino acids at $\alpha$ C terminal end	
Deletions		
Name	Deletion	Functional Abnormalities*
Freiburg <sup>159</sup>	$\beta$ 23 (B5) Val	UMe
Gun Hill	5 amino acids in segment $\beta$ 91-97 (F7-FG4)	Ue
Koellicker	$\alpha$ 141 (HC3) Arg (split off by plasma carboxypeptidase)	
Leiden	$\beta 6$ or 7 (A3 or A4) Glu	Uc
Niteroi <sup>145</sup>	$\beta$ 42-44 (CD1-CD3)	U
St Antoine <sup>42</sup>	$\beta$ 74-75 (E18 19) Gly Leu	
Tochigi <sup>45</sup>	$\beta$ 56 59 (D7 E3)	U
Tours <sup>42</sup>	$\beta$ 87 (F3) Val	Ue
Tetramers		
Name	Structure	Functional Abnormalities
Augusta 1	$\beta_4^x$	
Augusta 2	$\beta_4^c$	
Bart's	$\gamma_4$	U
H	$\beta_4^s$	Ue

\*Abbreviations are the same as in Table 24-1

are produced in the heterozygous state, as in unstable hemoglobin disease, then the pattern of inheritance of the "disease" will be that of an autosomal dominant trait. If, on the other hand, only the homozygous condition is symptomatic, as in sickle cell anemia, a recessive inheritance pattern is observed and the heterozygous state is called the "trait." Whether symptomatic or not, the heterozygous state is detectable by means of hemo-

globin analysis. The expected inheritance patterns with various matings between heterozygous and homozygous individuals are given in Table 24-3.

A patient carrying two different abnormal hemoglobin genes is referred to as *doubly heterozygous*. The inheritance pattern in such situations may take one of two possible forms, depending on whether the two genes are alleles or non-alleles; i.e., whether they

Table 24-3. Expected Inheritance Pattern with Certain Matings

Parent A	Parent B	Expected Proportion of Children		
		Normal (%)	Heterozygous (%)	Homozygous (%)
Heterozygous	Normal	50	50	0
Heterozygous	Heterozygous	25	50	25
Homozygous	Normal	0	100	0
Homozygous	Heterozygous	0	50	50
Homozygous	Homozygous	0	0	100

 From Lee<sup>100</sup> courtesy of the author and Harper & Row

affect the same polypeptide chain or different ones (Table 24-4). For example, a man doubly heterozygous for Hbs S and C, both of which are  $\beta$ -chain defects and therefore allelic, passes one, and only one, of these genes along to his children.<sup>111</sup> All children, therefore, will be heterozygous for either Hb S or Hb C, but none will be normal and none will be doubly heterozygous. Non-allelic inheritance, when one gene affects the  $\alpha$ -chain and one the  $\beta$ -chain, is quite different, as has been observed in several families.<sup>119,123</sup> In such pedigrees, children of the doubly heterozygous patient may be normal, or they may inherit either or both abnormal genes.

Because of their close linkage,  $\delta$ -chain abnormalities tend to be inherited with a  $\beta$ -chain abnormality on the same chromosome (cis), but if on opposite chromosomes (trans) they will not be inherited together.<sup>93,112</sup>

### Hemoglobin Patterns Associated with Various Genetic Constitutions

The homozygous state has been observed with hemoglobins S, C,<sup>120</sup> D Punjab,<sup>85</sup> E,<sup>102</sup> and G Accra,<sup>88</sup> all of which are  $\beta$ -chain variants, and with J Tongariki,<sup>81</sup> an  $\alpha$ -chain variant. Such patients are unable to make hemoglobin A. Hemoglobins A<sub>2</sub> and F are qualitatively normal in homozygous  $\beta$ -chain disorders, but increased amounts of HbF often persist into adult life and the proportion of HbA<sub>2</sub> often is slightly increased.<sup>94</sup> In homozygous  $\alpha$ -chain disease, the hemoglobin A<sub>2</sub> component is qualitatively abnormal since it contains the abnormal  $\alpha$ -chain.

Most of the abnormal hemoglobins have been observed in the heterozygous state. In such patients, HbA usually predominates and the abnormal hemoglobin constitutes less than 50% of the total, usually about 20 to 40%. Exceptions to this rule include many

 Table 24-4. Allelic and Non-Allelic Inheritance Patterns in Doubly Heterozygous Individuals<sup>111,119</sup>

Patient	Spouse	Expected Proportion of Children		
		Normal	Heterozygous	Doubly Heterozygous
Two genes affecting same chain (allelic), eg. HbS and C	Normal	0	100% (50%S 50%C)	0
Two genes affecting different chains (non-allelic), eg. HbS Hopkins II	Normal	25%	50% (25%S, 25% Hopkins II)	25%

members of the hemoglobin J group, in which more than 50% of the hemoglobin may be abnormal, possibly because the abnormal fraction is contaminated with hemoglobin A<sub>2</sub><sup>116</sup> (Chapter 4, page 172). The reasons for the usually low proportion of abnormal hemoglobin are not known, but one possibility is that the abnormal chain is synthesized less rapidly than the normal.<sup>83</sup> Another is that cells with varying proportions of the normal and abnormal hemoglobins are released from the marrow and that those with the higher proportions of abnormal hemoglobin are more rapidly destroyed than the others.<sup>48</sup> In unstable hemoglobins, precipitation and splenic removal also may reduce the circulating amount of abnormal hemoglobin.

As a general rule, the proportion of abnormal hemoglobin in heterozygotes with  $\alpha$ -chain defects is lower than in those with  $\beta$ -chain defects. In the former, the abnormal hemoglobin constitutes an average of 23% of the total, whereas in the latter an average of 38% has been found.<sup>116</sup> This phenomenon is observed even when  $\alpha$ - and  $\beta$ -variants have homologous substitutions, such as those in Hb J Oxford ( $\alpha$  A13 Gly  $\rightarrow$  Asp) and Hb J Baltimore ( $\beta$  A13 Gly  $\rightarrow$  Asp). This fact might be explained by the presence of a total of four structural  $\alpha$ -genes (see above), only one of which is a mutant in the usual heterozygote.

In patients heterozygous for  $\beta$ -chain variants the proportion of HbA<sub>2</sub> may be slightly increased,<sup>84,121</sup> but the HbA<sub>2</sub> and HbF fractions are qualitatively normal. In contrast,  $\alpha$ -chain abnormalities affect the minor hemoglobin fractions as well as the major. Thus, in infants, as many as six hemoglobins may be detected: Hb A, Hb A<sub>2</sub>, and Hb F, along with three corresponding abnormal hemoglobins containing the mutant  $\alpha$ -chain.<sup>122</sup>

The hemoglobin pattern in doubly heterozygous individuals depends on whether the two abnormal genes are alleles or not. When they are allelic, as in hemoglobin SC disease, no hemoglobin A is made. Two abnormal hemoglobins (eg, HbS and HbC) are found in the circulation in approximately equal amounts and, in addition, there may be some

compensatory increase in hemoglobin F. In non-allelic double heterozygotes, four variants of adult hemoglobin will circulate. Such individuals can synthesize  $\alpha^A$ ,  $\alpha^X$ ,  $\beta^A$ , and  $\beta^Y$ -chains (X and Y superscripts indicating the mutant chains) and can form the following four hemoglobins:  $\alpha^A\beta^A$  (HbA),  $\alpha^X\beta^A$  (HbX),  $\alpha^A\beta^Y$  (HbY) and  $\alpha^X\beta^Y$  (HbXY). Such a pattern has been reported in a number of instances.<sup>82,96,113,114</sup>

### Genetic Events Leading to Abnormal Hemoglobins

The most common genetic alteration resulting in an abnormal hemoglobin molecule is presumed to be a *point mutation*, ie, the substitution of a single DNA nucleotide base for another. This alters the genetic code, often in such a way that an amino acid change occurs in the resulting globin. When the amino acid substitutions in the reported human hemoglobin variants were examined in relation to the genetic code, all such variants were found to be explainable by a change in only one of the three bases coding for each amino acid.<sup>48</sup> Substitutions that cannot occur by such an alteration have not been observed. For example, the substitution Asp  $\rightarrow$  Lys does not occur because neither of the codons for aspartic acid (GAC, GAU) can change to a codon for lysine (AAA, AAG) without altering two bases.

Only one possible exception to the one-base change rule has been noted.<sup>32,48</sup> In HbM Milwaukee, the change at  $\beta 67$  is Val  $\rightarrow$  Glu; in Hb Bristol it is  $\beta 67$  Val  $\rightarrow$  Asp. None of the known valine codons (GUU, GUC, GUA, and GUG) could change to both a glutamic acid codon (GAA, GAG) and an aspartic acid codon (GAU, GAC) by means of one-base changes. Thus, two possibilities exist: (1) either HbM Milwaukee or Hb Bristol occurred as a result of a double mutation, or (2) the  $\beta 67$  valine codon in normal globin mRNA is variable.

Lehmann and Carrell have estimated the one-base mutation rate at the hemoglobin locus at 1 in  $10^4$  individuals per generation.<sup>48</sup> At least 20 families have been recorded in which a spontaneous mutation probably occurred.<sup>32</sup>

Two types of point mutations have been defined. A *transition* is a substitution of a pyrimidine for another pyrimidine or a purine for another purine. A *transversion* is a substitution of a purine for a pyrimidine or vice versa. The amino acid changes produced by transversions tend to result in more radical alterations in protein structure than those occurring with transitions. If mutations occurred at random, twice as many transversions as transitions should be observed. However, the number of human hemoglobin variants resulting from transitions are about equal to those resulting from transversions.<sup>48</sup> If only substitutions affecting the molecular surface are tabulated, the expected 2:1 ratio is found, but changes in the molecular core are only rarely produced by transversion. Purine transitions are more common than pyrimidine transitions, and the most common of all point substitutions is adenine → guanine in DNA (guanine → adenine in RNA). Bunn found that 25 of 114 known hemoglobin variants arose from this mutation.<sup>32</sup> The reasons for the non-random nature of these genetic events are not entirely clear.

The mutation leading to Hb Constant Spring might have been a simple point substitution in a "stop" codon.<sup>85</sup> This unusual hemoglobin has 31 residues added to the normal  $\alpha$ -chain, and the first "extra" residue is glutamic acid (codons: GAA, GAG). The glutamic acid residue could have arisen from a one-base change in either of the two codons (UAG, UAA) that code for chain termination. Hemoglobin Tak, which has extra residues on the end of the  $\beta$ -chain, cannot be explained by a similar change; the first extra amino acid is threonine, and its codons (ACC, ACU, ACA, AGG) cannot arise from any of the "stop" codons by a one-base change.<sup>90</sup> It has been suggested that the normal  $\beta$ -chain may be longer than 146 amino acids when first synthesized and that one or more residues are lost by hydrolysis. If so, Hb Tak might then represent a stop codon mutation.

Genetic crossovers between chromosomal pairs also have the potential for generating abnormal hemoglobins. The usual crossovers occur at identical loci on the chromosome

pairs; these are called "homologous" or "equal" crossovers. Such a crossover in an individual carrying genes for HbS ( $\beta^6$  Val) and Hb Korle Bu ( $\beta^{73}$  Asn) is a possible way to form the gene for Hb C Harlem, carrying both substitutions.<sup>99</sup> Unequal or non-homologous crossing over (chromosomal mutation) has the potential for producing extensive changes in structural genes, such as deletion of a portion of the gene, as well as an addition to it or a fusion of genes at the crossover sites.<sup>101</sup> The hemoglobins with single or multiple deletions (Table 24-2) probably arose in this way since a minimum of three nucleotide bases must have been deleted. Furthermore, the "fusion genes" resulting in hemoglobins of the Lepore, Miyada, or Kenya type (Table 24-2) almost certainly were formed by unequal crossing over (see Fig. 26-9, page 874), and a similar mechanism may have resulted in Hb Hopkins 2-II<sup>106</sup> (Table 24-2).

One unusual hemoglobin may have been induced by a "frameshift" in the genetic code. If a single DNA base is added or deleted, all tripler codons distal to the change will be altered. The effects of such a change are most drastic when they occur toward the beginning of the polypeptide chain, since everything beyond the frameshift is "nonsense." Toward the end of the chain, however, the change might be tolerated. The amino acid sequence in Hb Wayne includes altered terminal residues and added amino acids,<sup>117</sup> and inspection of the possible codons supports the hypothesis that a frameshift due to a base deletion could account for the observed sequence.

The genetic mechanisms resulting in thalassemias and the formation of the tetrameric hemoglobins are discussed in Chapter 26.

### Molecular Pathology and the Functional Classification of Abnormal Hemoglobins

The careful construction by Perutz and co-workers of a three-dimensional model of the hemoglobin molecule<sup>109</sup> (Chapter 4) has made it possible to visualize the effects of amino acid substitution on molecular func-



tion.<sup>108</sup> The Perutz model demonstrates that the water-free, molecular core is stabilized by nonpolar interactions of the Van Der Waals type. Amino acids with polar side chains are completely excluded from the interior and are found only on the molecular surface, where they interact with water, rendering the molecule soluble. The heme group is covalently bound at the F8 histidine, but also forms about 60 contacts with nonpolar amino acids in the heme crevice, thereby contributing to the stability of the tertiary structure. This nonpolar environment makes possible the association of heme iron with oxygen without significant methemoglobin formation. The contacts between the four polypeptide chains in each molecule were also evaluated from the model, and found to be of two kinds. The larger contact, called  $\alpha_1\beta_1$ , is formed from nonpolar interactions among 34 amino acids; the smaller,  $\alpha_1\beta_2$ , involves only 19 amino acids (see Chapter 4). With oxygenation, the  $\alpha_1\beta_2$  contact undergoes significant alteration, a movement of about 0.7 nm (7 Å) or so taking place, producing so-called heme-heme or subunit interaction. This and other stereochemical changes involved in oxygen binding have been discussed by Perutz.<sup>107</sup> With this general background, the effects of substitution at certain molecular sites can be un-

derstood and a functional classification<sup>91</sup> of the abnormal hemoglobins can be made (Table 24-5).

Substitutions at the *molecular surface* usually are innocuous because they are not likely to affect tertiary structure, heme function, or subunit interaction. Indeed, most functionally normal hemoglobin variants, which produce no symptoms, are characterized by substitution at surface positions. Most of them have been discovered by means of hemoglobin electrophoresis in population screening studies and, therefore, result from substitutions that change the net charge of the molecule. Some surface substitutions result in hemoglobin molecules that tend to polymerize or *aggregate*. It is likely that such mutations create a site on the molecular surface that can interact with a complimentary site on an adjacent molecule. The exact nature of these molecular interactions is unknown, but some hypotheses are discussed in Chapter 25. The aggregating hemoglobins, of which Hb S is the prototype, tend to crystallize or polymerize intracellularly with consequent distortion of cell shape, reduced cell deformability, hemolysis, and impaired microvascular circulation.

A few abnormal hemoglobin variants exhibit *reduced structural stability*, as evidenced

Table 24-5. Functional Classification of Abnormal Hemoglobins

<i>Functional Abnormality</i>	<i>Location of Substitution</i>	<i>Clinical Abnormality</i>	<i>Example</i>
None	Surface	None	Hb G Philadelphia
Aggregation with reduced solubility	Surface	Hemolytic anemia (homozygous)	Hb S (Chapter 25)
Instability with reduced solubility	Internal nonpolar residues	Hemolytic anemia (heterozygous)	Hb Köln (Table 24-8)
Methemoglobinemia	Proximal (F8) or distal (E7) histidine	Cyanosis	Hb M (Chapter 31)
Increased oxygen affinity	$\alpha_1\beta_2$ Contact or $\beta$ C terminal	Erythrocytosis	Hb Chesapeake (Chapter 30)
Decreased oxygen affinity	Near heme and $\alpha_1\beta_2$ contact	Cyanosis	Hb Kansas (Chapter 31)

by (1) their tendency to precipitate intracellularly, forming inclusion bodies, and (2) their precipitation with mild heating (eg, to 50° C). Most of these unstable hemoglobins result from neutral (uncharged) substitutions affecting internal, nonpolar residues. Many of these substitutions affect residues that contact the heme group<sup>18</sup>; thus, heme-globin bonding is reduced, water may gain access to the heme pocket, and heme may drop out of the molecule. Heme-free normal globin<sup>89</sup> and partially heme-free hemoglobin ( $\alpha_2^{\text{heme}}\beta_2$ )<sup>98</sup> are, themselves, unstable. Some unstable hemoglobins are characterized by a marked change in conformation of the entire molecule, especially those hemoglobins with deletions (eg, Hb Gun Hill), and those in which proline has been inserted into a helical segment, a change which disrupts or bends the helix (eg, Hb Bibba,  $\alpha 136$  (H19) Leu  $\rightarrow$  Pro). The Van der Waals forces upon which structural stability depend are greatly affected by molecular dimensions; therefore, the altered dimensions of the substituted amino acid can have far-reaching consequences. It is likely that the insertion of a changed amino acid into the nonpolar core would result in a completely nonviable molecule<sup>48</sup> unless the charge can be stabilized by internal salt formation, as in Hb Wien, or the charged group can be accommodated on the molecular surface, as in hemoglobins Sogn, Riverdale-Bronx, Shepherds' Bush, and Ann Arbor.<sup>217</sup>

Substitution of a tyrosine for either the proximal (F8: $\alpha 87$ ,  $\beta 92$ ) or distal (E7: $\alpha 58$ ,  $\beta 63$ ) histidine produces an "M" hemoglobin (Chapter 31). These substitutions allow an ionic bond to form between heme iron and the phenolic oxygen of tyrosine, thereby stabilizing iron in the nonfunctional, ferric state. In Hb M Milwaukee ( $\beta 67$  [E11] Val  $\rightarrow$  Glu), a similar ionic bond forms with the glutamic carboxyl group. Such heme groups cannot bind oxygen, and affected patients have methemoglobinemia and cyanosis.

A number of hemoglobins that exhibit increased oxygen affinity, often leading to erythrocytosis, have been described (Table 30-3). Most of the substitutions producing this abnormality occupy an  $\alpha_1\beta_2$  contact

point, where they impair subunit (heme-heme) interaction. A few have been near the C-terminal end of the  $\beta$ -chain, where they interfere with the alkaline Bohr effect, with 2,3 DPG binding, or with the formation of salt bridges that tend to stabilize hemoglobin in the deoxy, low oxygen affinity state (Chapter 4, page 176).

In hemoglobins Kansas, Seattle, and Hammersmith, oxygen affinity is reduced sufficiently to produce cyanosis. A mild reduction in oxygen affinity without cyanosis is found in a few others, such as HbE and certain unstable hemoglobins (page 816). The substitution in Hb Kansas ( $\beta 102$  [G4] Asn  $\rightarrow$  Thr) occurs at a residue which forms part of the  $\alpha_1\beta_2$  contact and which also contacts the heme group. A hydrogen bond normally serving to hold dimers together cannot form; hence this hemoglobin easily splits into dimers. The reduced oxygen affinity of Hb Seattle remains unexplained.

## Laboratory Approach to Abnormal Hemoglobin Identification

### Electrophoresis

Hemoglobin electrophoresis is the single most useful laboratory procedure for the detection and identification of abnormal hemoglobins. This technique separates proteins according to charge; thus, its principal limitation is its inability to detect amino acid substitutions that do not alter charge, such as those found, particularly, in certain of the unstable hemoglobins and the hemoglobins with increased oxygen affinity.<sup>50a,57</sup> It has been calculated that 2200 abnormal hemoglobins can theoretically occur as the result of single amino acid substitutions and that only about one third of these would have an abnormal charge.<sup>48</sup> That most presently known hemoglobins, with the exceptions noted, are electrophoretically abnormal is explained by the fact that electrophoresis was used as the screening method whereby many of them were detected.

Moving boundary electrophoresis in a Tiselius apparatus was the method used by Pauling et al to detect Hb S.<sup>8</sup> Because of the expense and bulk of such apparatus, this method soon was replaced in many laboratories with various techniques for zone electrophoresis, by means of which protein components move as separate zones on various types of supporting media. A wide variety of such methods are available. These differ from one another in the nature of the supporting medium, the buffer, the apparatus, and other methodologic details. Techniques based on filter paper as a supportive medium were the first to be used in routine laboratories<sup>146</sup>; however, because of relatively poor resolution, inability to demonstrate the HbA<sub>2</sub> fraction, and difficulty in quantitation, this method lost favor. Electrophoresis on starch gel rapidly became the method of choice in research laboratories because of its sensitivity and superior definition of zones.<sup>169,199</sup> Most of the available information regarding electrophoretic properties of the hemoglobin variants has been obtained with the starch gel method. Nevertheless, it is too cumbersome and the migration time too long for most routine laboratories. The simplest and most popular routine methods employ cellulose acetate membranes,<sup>144,163,166</sup> for which many types of electrophoresis equipment are available commercially. With this method, hemoglobin electrophoresis can be completed in 30 to 120 minutes. The technique can be made quantitative by elution of zones and spectrophotometric measurement of the hemoglobin that they contain or by spectrophotometric scanning of stained and clarified membranes.

Routine electrophoresis for screening purposes is carried out at alkaline pH (usually 8.6 to 9.1) with tris-EDTA-borate (TEB) buffer or with a "discontinuous" buffer system (TEB in the anodal well and barbital buffer in the cathodal well). Under these conditions, abnormal hemoglobins can be divided into six major groups, depending on their mobility in relation to simultaneously analyzed "marker" hemoglobins—ideally, hemoglobins A, S, and H<sup>169</sup> (Fig. 24-1 and Table 24-6). The six groups are named ac-

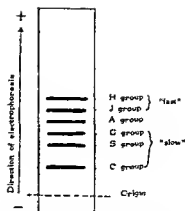


Fig 24-1. Electrophoretic mobility of six hemoglobin groups at pH 8.6. The groups are named for the principal hemoglobin with that mobility. For others in each group see Table 24-6.

cording to the principal hemoglobin in the group. With very careful technique and additional "markers," even further differences in mobility may be observed (Fig. 24-2), but such fine distinctions are not possible in most clinical laboratories.

Table 24-6. Electrophoretic Mobility of Certain Hemoglobins<sup>169</sup>

Group*	Mobility	Principal Hemoglobins
C	Slower than S. HbA <sub>2</sub> is a marker	C, E, A <sub>2</sub> , O, F Alexandra
S	Slow. Hb S is a marker	S, D Stanleyville-2 Lepore
G	Slower than A. faster than S	G, L, P, Q
A	HbA is a marker	A M, F†, unstable Hb s, certain Hb s with increased O <sub>2</sub> affinity‡
J	Faster than A, slower than H	J, K, N, Norfolk
H	Very fast. HbH is a marker	H, I Bart's

\*Named for principal hemoglobin in the group  
 †HbF is slightly slower than HbA on starch gel  
 ‡See Tables 24-8 and 30-3

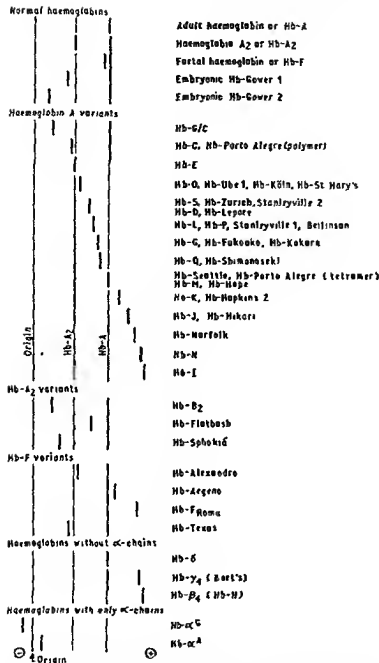


Fig 24-2. Relative electrophoretic mobility of human hemoglobin variants at pH 8.6 on starch gel (From Huehns and Shooter,<sup>199</sup> courtesy of the authors and Journal of Medical Genetics)

Electrophoresis in agar gel at acid pH (usually in citrate buffer, pH 6.2) is a useful procedure for further fractionation of some of the groups described above.<sup>143,165,170</sup> It is not a satisfactory screening technique because it cannot distinguish many abnormal hemoglobins from HbA. However, it can separate

the C group into three fractions: C, O, and E plus A<sub>2</sub>. Also, the method can distinguish Hb S from HbD, HbF from HbA, hemoglobins Little Rock, Ranier and Bethesda from HbA,<sup>21</sup> and HbH from HbL.

The M hemoglobins can be separated from HbA by converting all hemoglobin to meth-

moglobin with ferricyanide, then performing electrophoresis at pH 7.0, usually on starch gel.<sup>151</sup>

## Tests Based on Altered Physical or Chemical Properties

### Tests for Hb S

Hemoglobin S may be detected by the sickling phenomenon and also by methods that measure hemoglobin solubility. These are discussed in Chapter 25 (page 838). In particular, Hemoglobin SD disease can be distinguished from sickle cell anemia by a differential solubility test.<sup>157</sup>

### Tests for Hb F

Hemoglobin F is most easily quantified by means of the alkali denaturation test.<sup>141,147,148</sup> Hb A is denatured by a one-minute exposure to a standardized solution of KOH (pH = 12.7) at room temperature, whereas Hb F resists denaturation under these conditions. The denatured hemoglobin is precipitated with ammonium sulfate and any hemoglobin remaining in the solution is measured spectrophotometrically. The test can measure Hb F in amounts greater than 0.5% with a high degree of accuracy. Hb Bart's and Hb Ranier also resist alkali denaturation.<sup>22</sup>

The cellular distribution of Hb F can be evaluated with a differential staining technique.<sup>160,168</sup> Hemoglobin A is eluted from red cells on a fixed blood film with a citric acid-phosphate buffer (pH 3.2), but Hb F remains in the cells. If, after such treatment, the slides are stained with hematoxylin and erythrosin,<sup>148</sup> cells containing large amounts of Hb F stain darkly, whereas cells with little or no Hb F appear unstained and empty. In most of the hemoglobinopathies and thalassemias, all circulating Hb F is found in a single cell line, thus, two distinct populations will be seen with the described procedure, and the size of the darkly staining population is proportional to the amount of Hb F. The only well-documented exception to this generalization is hereditary persistence of

Hb F (Chapter 26, Fig. 26-10) in which the Hb F is evenly distributed among the erythrocytes.

### Tests for Unstable Hemoglobins

The heat denaturation test<sup>149</sup> has been a useful screening test because many unstable hemoglobins are not detected by routine electrophoretic procedures. Clear hemolysates from the patient and a normal control are incubated with an equal amount of 0.1M phosphate buffer, pH 7.4, at 50° C for one to two hours, and the preparations are examined at intervals for precipitation. Alternatively, somewhat higher temperatures (60° to 65° C) may be used for shorter periods.<sup>31,40</sup> Normal hemoglobin remains in solution much longer under these conditions than the unstable hemoglobins (Table 24-8). Usually, an abnormal result is apparent on visual inspection of the amount of precipitate in the tube. The quantity precipitated can be measured by analyzing the supernatant solution for hemoglobin before and after a two-hour incubation at 50° C and calculating the difference. The rate of precipitation can be more precisely estimated by removing aliquots at five-minute intervals during a 60° or 65° C incubation and analyzing as described above. A graph constructed from such data demonstrates dramatic differences between a normal hemolysate and one containing an unstable hemoglobin (Fig. 24-3).

Another promising method for detecting unstable hemoglobins is the isopropanol precipitation test of Carrell and Kay.<sup>145</sup> In this test, 0.2 ml of a freshly prepared hemolysate is added to 2.0 ml of a 17% (by volume) solution of isopropanol in 0.1M Tris-HCl buffer, pH 7.4, and incubated at 37° C. With hemolysates from seven different unstable hemoglobins (Christchurch, Sydney, Köln, Wien, Niteroi, Shepherds' Busb,<sup>145</sup> and Southampton<sup>42</sup>), unequivocal clouding was observed at five minutes and a flocculent precipitate had formed within 20 minutes. With 200 hemolysates from normal subjects, no precipitation was observed until after 40 minutes. With hemolysates containing Hb H, a slight opacity was observed at 10 minutes.

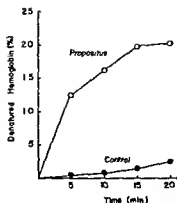


Fig 24-3. The heat denaturation test in a patient with Hb Istanbul (propositus) as compared with control. The test was performed at 60°C (From Aksoy et al<sup>23</sup> courtesy of the authors and *Journal of Clinical Investigation*.)

Precipitated material can be used for further chemical analysis.

Inclusion bodies may sometimes be demonstrated with supravital dyes (eg, methyl or crystal violet) in red cells containing unstable hemoglobins (page 813). These so-called Heinz bodies are not specific for unstable

hemoglobin disease, since they also may appear in glucose 6-phosphate dehydrogenase deficiency and related disorders (Fig. 24-4). Furthermore, the inclusions usually are not detected in patients with unstable hemoglobin disease unless the spleen has been removed. Various measures induce the appearance of such inclusions in nonsplenectomized patients, such as 24- or 48-hour incubation at room temperature<sup>149</sup> or the addition of sodium nitrate<sup>148</sup> or acetylphenylhydrazine.<sup>142</sup> In general, however, the test is less specific and less sensitive than the heat or isopropanol denaturation tests. Prolonged incubation with brilliant cresyl blue is a useful procedure for distinguishing Hb H from Hb I because only Hb H forms inclusions. The Hb H inclusions appear as fine, dust-like particles rather than the larger Heinz bodies.<sup>164</sup>

### Chromatography

Separation of hemoglobins on chromatographic columns has been accomplished with

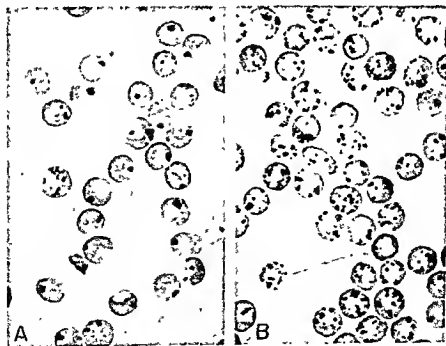


Fig 24-4. Heinz-body formation in blood of persons sensitive to primaquine (B) compared with that in nonsensitive individuals (A). The red corpuscles were incubated with 1.0 g/l acetylphenylhydrazine solution for four hours. Wet preparations stained with crystal violet (Magnification, 1300×) (From Beutler et al,<sup>142</sup> courtesy of the authors and *Journal of Laboratory and Clinical Medicine*.)

various ion-exchange resins, including diethylaminoethyl (DEAE) cellulose,<sup>153</sup> DEAE Sephadex,<sup>154</sup> carboxymethyl (CM) cellulose,<sup>156</sup> CM Sephadex,<sup>150</sup> and Amberlite IRC-50<sup>155</sup> (see Fig. 4-15, page 173). Although mainly suited for research purposes, particularly for purification of hemoglobins prior to chemical analysis, chromatographic techniques also have been adapted for use as a diagnostic method in some laboratories.<sup>169</sup> For example, they can serve as an alternative to acid-agar gel electrophoresis for such purposes as separating Hb A from Hb F, Hb H from Hb I, and C from O and E. A chromatographic method may also be used to separate and quantitate Hb A<sub>2</sub> in the presence of Hb C.<sup>94</sup>

### Hybridization

Hybridization is used to determine which of the polypeptide chains in an abnormal hemoglobin contains the substituted amino acid. The  $\alpha$ - and  $\beta$ -chains are dissociated at low pH and recombined with similarly dissociated chains of canine hemoglobin. The products are analyzed by starch gel electrophoresis. Four hemoglobins will be found—human, canine, and two hybrids formed from the  $\alpha$ -chains of one species and the  $\beta$ -chains of the other. The abnormal chain can be identified by the altered position of the hybrid containing it.<sup>152</sup>

### Peptide Analysis

The first step in defining the precise abnormality in an abnormal hemoglobin consists of breaking the molecule down into smaller peptides. Usually this is accomplished by proteolysis with trypsin, a procedure that cleaves the protein at lysyl and arginyl residues, thereby forming 14 peptides from the normal  $\alpha$ -chain and 15 from the  $\beta$ -chain. Some of these peptides remain insoluble (the "core"), unless trypsin hydrolysis is preceded by S-aminoethylation, which allows trypsin cleavage also to occur at cysteine residues.<sup>158</sup>

The tryptic peptides are then separated from one another. Most frequently, this has

been accomplished by applying the mixture to filter paper and performing high-voltage electrophoresis along one axis and chromatography along the other. Each peptide then occupies a unique position on the filter paper, and the resulting pattern, stained with ninhydrin, is called a "fingerprint" or peptide map<sup>6</sup> (Fig. 24-5). The peptide containing the abnormal amino acid is identified by its changed position. Alternatively the peptides can be separated by automatic column chromatography procedures, the abnormal peptide being characterized by an altered elution volume<sup>158</sup> (Fig. 24-6).

Individual peptides may be eluted from the "fingerprint" or collected from the column and subjected to chemical analysis to determine the amino acid sequence, usually by the Edman degradation methods.<sup>162,167</sup>

Peptide analysis and amino acid sequencing are techniques suited only to a well-equipped research laboratory. They are used only when precise identification is required and especially when a possible new variant is detected. Nevertheless, at least one abnormal hemoglobin could not be detected by any means other than peptide analysis.<sup>57</sup>

### Prevalence<sup>202</sup>

The frequency of the abnormal hemoglobins varies considerably with geographic location and racial group. Four hemoglobins, S, C, D Punjab, and E, often are called the "common hemoglobins" because each affects millions of individuals.

Hemoglobin S is certainly the most common of all abnormal hemoglobins. It is found particularly in equatorial Africa in a broad zone extending from coast to coast<sup>202</sup> (Fig. 24-7). The highest incidence occurs in the eastern part of the continent, where 40 to 50% of the members of certain tribes are affected. A prevalence rate of 10 to 20% is common throughout other parts of the zone, but there is considerable variation from one tribe to another.<sup>201</sup> For example, only 2.9% of members of the Hamitic tribes carry the gene as compared with 19% of Bantus. Hemoglobin S also is found in Southern

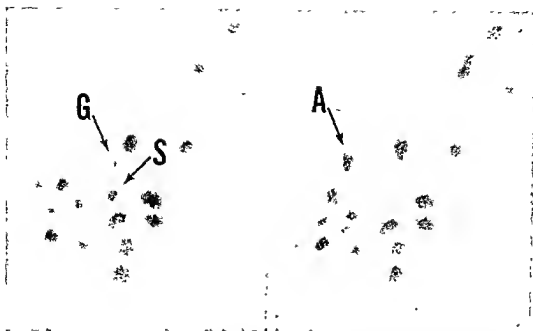


Fig. 24-5. Peptide map ("fingerprint") of tryptic digests of an equal mixture of Hb G San Jose and Hb S (left) compared with that of Hb A (right). The peptide from Hb A (indicated by the arrow) is absent from the pattern of G and S and is replaced by two abnormal peptides (indicated by arrows, one from G and the other from S) (Prepared by Dr. Robert L. Hill)

Turkey, Arabia, and India, predominantly in relatively isolated and primitive people.<sup>200</sup> It was suggested that the African and Indian populations inherited the gene independently from a common source in the Middle East. From this source, the gene may have been introduced into Africa via a land bridge that once connected the continents, and from there spread westward. Sickle cell trait also occurs in areas along the Northern Mediterranean shores (Fig. 24-7), especially in Greece where as many as 32% of the inhabit-

ants of certain villages exhibit the sickling phenomenon.<sup>195</sup>

The incidence of the sickle cell gene in migrant Negro populations probably reflects the African origin of these people. In the USA, the overall prevalence of the trait among Negroes is about 8% (Table 24-7), but there may be considerable variation in different parts of the country. A lower incidence is found in the north, possibly because of greater racial admixture, whereas in such isolated populations as the Gulla Negroes of

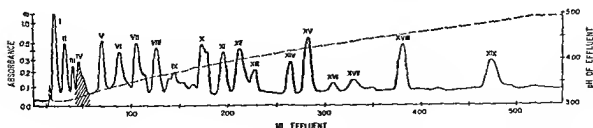


Fig. 24-6. Peptide analysis by automatic column chromatography of the tryptic hydrolysate of aminoethylated  $\beta$ -chains of Hb Freiburg. The solid line indicated absorbance at 570 nm of the ninhydrin reaction products of peptides. Each zone, numbered with Roman numerals, represents a single peptide. The shaded zone IV is the abnormal peptide; the pattern is otherwise identical to that of the normal  $\beta$ -chain. The pH of the effluent is shown as a dashed line. (From Jones et al.,<sup>139</sup> courtesy of the authors and Science)



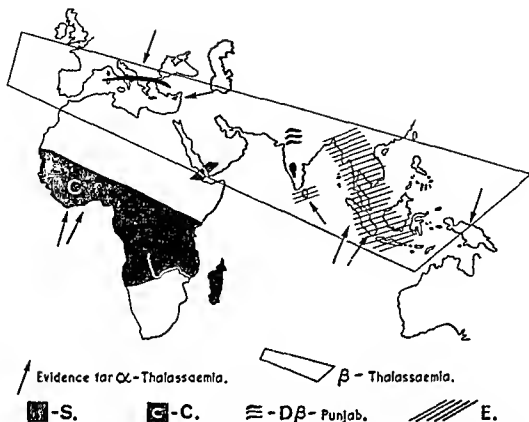


Fig 24-7 Geographic distribution of sickling trait  $\beta$ - and  $\alpha$ -thalassaemia, hemoglobins C and E and Hb-D $\beta$ -Punjab. In Western Africa Hb-C is found in addition to Hb-S, as indicated in the map (Specially prepared by Dr Hermann Lehmann)

South Carolina, the prevalence of Hb S approaches 20%.<sup>202</sup> The frequency of the gene in Central and South America and in the West Indies is similar to that in North America. Additional prevalence rates in various countries have been assembled by the World Health Organization<sup>209</sup> and by Livingstone.<sup>202</sup>

Compared with Hb S, Hb C is found in a much smaller and more sharply demarcated geographic zone in Western Africa (Fig. 24-7). The greatest prevalence is in Northern Ghana, where 28% of the population harbor the gene.<sup>196</sup> The frequency declines sharply to the south, east, and west. Within Nigeria, the River Niger seems to have acted as a barrier to eastward spread. In the USA, about 3% of the Negro population carries the Hb C gene (Table 24-7).

Hemoglobin E is most prevalent in South-

east Asia,<sup>202</sup> affecting over 50% of the population in eastern Thailand (Surin Province) and from 20 to 45% in other parts of Thailand and in Cambodia, Laos, and Burma. A somewhat lower prevalence, 3 to 8%, is found in Vietnam. From the mainland, the gene spread southward to parts of Indonesia, but the incidence is low in India to the west and in China to the northeast.

Hemoglobin D Punjab is found in the greatest frequency (2%) among the Sikhs of the Punjab in India (Fig. 24-7) as well as in nearby Gujarat (1%)<sup>193</sup> and in Iran. It has also been found in American Negroes (0.4% in North Carolina<sup>194</sup>) sporadically in Caucasians throughout the world, and in American Indians.<sup>193</sup>

The high prevalence rates of the "common" hemoglobins as well as thalassemia (Chapter 26) and G6PD deficiency (Chapter

**Table 24-7. Estimated Frequency of the Most Common Hemoglobinopathies and  $\beta$ -Thalassemia in American Negroes<sup>205</sup>**

Disorder*	Frequency	
	At Birth	All Ages
Heterozygous conditions	%	%
Hb S trait	8.0	—
Hb C trait	3.0	—
$\beta$ -Thalassemia minor	1.5	—
HPFH* trait	0.1	—
Homozygous conditions	per 10 <sup>5</sup>	per 10 <sup>5</sup>
Sickle cell anemia	160.0	53
Hb C disease	22.5	—
Thalassemia major	5.6	—
HPFH*	0.025	—
Doubly Heterozygous conditions	per 10 <sup>5</sup>	per 10 <sup>5</sup>
S-C Disease	120.0	80
S-Thalassemia	60.0	30
S-HPFH*	4.0	—
C-Thalassemia	22.5	—
C-HPFH*	1.4	—
Thalassemia HPFH*	0.75	—

\*HPFH hereditary persistence of fetal hemoglobin

23) suggest that the deleterious effects of the homozygous states of these disorders are counterbalanced by the selective advantage that they provide to heterozygous individuals ("balanced polymorphism").<sup>206</sup> By this mechanism, the gene frequency increases in the population despite the adverse effects on survival of homozygous individuals, because the heterozygote is "fitter" in the genetic sense than a normal subject. Most of the studies of this phenomenon have dealt with the role of malaria, especially *falciparum* malaria, as the selecting agent and with the possible resistance to malaria offered by subjects heterozygous for the above disorders.

The "malaria hypothesis" is best established with respect to hemoglobin S. The geographic distributions of *falciparum* malaria and Hb S coincide remarkably,<sup>199</sup> and the frequency of sickle trait is correlated with the endemicity of malaria in many tribes.<sup>192</sup> Furthermore, a lower rate of parasitization of the blood is found in subjects with sickle

trait<sup>191,207</sup> even when they are deliberately inoculated with the parasite,<sup>192</sup> and the mortality rate from cerebral malaria is much lower in children with the trait than in those free of Hb S.<sup>204,207</sup> The mechanism of malarial resistance has not been established, but the most likely hypothesis is that the invaded cells adhere to vessel walls where they become deoxygenated and assume the sickled shape, which in turn leads to their destruction by phagocytosis.<sup>203</sup> When red cells are used in in vitro culture systems, growth occurs as well in Hb S cells as in normal cells.<sup>207</sup>

Most other hemoglobin variants are rare.<sup>202</sup> Many have been reported in a single individual or in a single family. Occasionally, in isolated populations, a minor variant may attain considerable prevalence; for example, Hb J Tongariki was found to affect nearly 10% of the population on an island in the Pacific.<sup>197</sup> In a survey of 8000 Europeans (5000 Danish, 3000 British), 11 persons with electrophoretically identifiable abnormal hemoglobins were detected.<sup>208</sup> Since only about one third of hemoglobin variants would be detected by this technique (page 805), and also allowing for the fact that certain substitutions are "forbidden" since they would produce a nonviable molecule, it may be calculated that the overall prevalence of abnormal hemoglobins in Europeans may be about 0.5%.<sup>48</sup>

## Unstable Hemoglobin Disease<sup>221,247</sup>

### (Congenital Heinz-Body Hemolytic Anemia)

In 1890, Heinz described the staining characteristics of the red cell inclusions that now bear his name.<sup>230</sup> For the next 60 years, Heinz bodies were considered to be solely a manifestation of exposure to various kinds of toxins.<sup>246</sup> Their relation to primaquine-induced hemolytic anemia, in particular, was established in the 1950's (Chapter 23). In 1952, Cathie reported a patient in whom similar inclusions were found in the absence of toxic exposure.<sup>224</sup> In this patient, a sple-

nectomy had been performed because of severe, congenital hemolytic anemia. This congenital form of Heinz-body anemia was more fully delineated by Lange and Akeroyd<sup>236</sup> and by Schmid et al.<sup>212</sup> who reported cases similar to Cathie's and observed the association with the urinary excretion of a brown, dipyrrolic pigment. Schmid's patients, being father and son, provided evidence that the disease was familial.

The first suggestion that an abnormal hemoglobin might be implicated in pathogenesis was made by Scott and coworkers, who found an electrophoretically abnormal hemoglobin component in a patient with congenital Heinz-body hemolytic anemia.<sup>213</sup> Their suggestion was supported by the discovery that Hb Zurich was associated with an inclusion-body hemolytic anemia, although only after exposure to sulfonamides.<sup>228</sup> That an abnormal hemoglobin might be present but electrophoretically "silent" was proposed by Dacie et al, who found a heat-labile hemoglobin fraction in their patients.<sup>149</sup> The discovery of Hb Köln and the determination of its molecular abnormality constituted final proof that congenital Heinz-body hemolytic anemia was a hemoglobinopathy.<sup>222</sup> Other unstable hemoglobins—Genova,<sup>53</sup> Sydney,<sup>223</sup> and Hammersmith<sup>225</sup>—were soon found to produce a similar picture. Now, over 40 unstable variants have been identified (Table 24-8), and a clearer picture of the spectrum of disease that they produce has emerged.

### Incidence

Despite the large number of reported variants (Table 24-8), unstable hemoglobin disease is considered to be rare. Most of the reports deal with single cases or single kindreds. A few unstable hemoglobins have occurred in two or a few unrelated families.<sup>224a</sup> *Hemoglobin Köln disease* is much more common than the others, however. At least 43 patients with this hemoglobin, in 11 apparently unrelated families, have been described.<sup>50b,149,222,226,227,229,231,232,237,238,240,245</sup>

Unstable hemoglobin disease is fully ex-

**Table 24-8. Clinical Classification of Unstable Hemoglobins<sup>235</sup>**

<b>A Producing severe hemolytic disease No clear response to splenectomy</b>	
Bibba	Sabine
Bristol	Savannah
Hammersmith	Southampton
Olmsied	
<b>B Producing moderate hemolytic disease Improvement after splenectomy.</b>	
Boras	Istanbul
Bucuresti	Santa Ana
Casper	Shepherds' Bush
Christchurch	Torino
Genova	Wien
<b>C Producing mild hemolytic disease except during crises</b>	
Ann Arbor	Peterborough
Bryn Mawr	Philly
Ferrara	Riverdale-Bronx
Freiburg	Rush
Gun Hill	Seattle
Hasharon	St Etienne
Köln	Sydney
Leiden	Tochigi
Louisville	Zürich
<b>D Producing no clinical or hemolytic abnormalities</b>	
Dakar	Sögn
Etobicoke	Tacoma
Hopkins-2	Toulouse
<b>E Insufficient data to classify</b>	
Khantoum	St. Louis
Niteroi	Tours

\*For definitions of severe moderate and mild see text. The structures of these hemoglobins are given in Tables 24-1 and 24-2.

pressed in heterozygous individuals; thus, the inheritance pattern is autosomal dominant. Homozygous disease has not been reported and would likely be lethal. In at least 14 instances,<sup>239</sup> both parents of an affected individual were normal, indicating that a spontaneous mutation had occurred. This type of pedigree was particularly common with the variants producing severe or moderately severe disease, possibly because patients with severe disease are less likely to reproduce.

### Clinical Manifestations and Degree of Anemia

Unstable hemoglobin disease varies considerably in severity. A classification has been

proposed<sup>235</sup> in which the reported variants are divided into four groups according to the intensity of the associated hemolytic process and the response to splenectomy (Table 24-8). With seven variants, the disease was considered *severe*. Hemolytic anemia usually became apparent in infancy, and the blood hemoglobin level ranged from less than 4 to about 8 g/dl. The reticulocyte count was reported to be very high, often 50% or more, but such values may be falsely high.<sup>235,247</sup> Splenectomy, followed by no clinical improvement, had been performed in all but one<sup>42</sup> of these patients.

In a second group of 10 variants, disease was *moderate* in severity. The disorder often was not detected until late in childhood or during adolescence. Often, symptoms of cholelithiasis, episodic jaundice, splenomegaly, or a hemolytic crisis were the presenting manifestations. Prior to splenectomy, the blood Hb averaged about 9 g/dl (range 6 to 11 g/dl) with 4 to 20% reticulocytes. After splenectomy, anemia was mild or absent, but reticulocytosis persisted.

*Mild* hemolytic disease has been associated with the majority of unstable variants. In the steady state, anemia was absent or mild, but the reticulocyte count often ranged from 4 to 10%. Splenomegaly was not a usual finding in this group. Many of the patients were detected because of a "crisis" characterized by an abrupt increase in the degree of anemia. Crises in either mild or moderate disease appeared to be precipitated by episodes of infection; less often, by ingestion of certain drugs. Drug-induced hemolytic anemia was best documented in the patients with hemoglobins Zürich,<sup>228</sup> Torino,<sup>239</sup> Peterborough,<sup>45</sup> and Shepherds' Bush,<sup>63</sup> and the offending agents were sulfonamides. Whether drug sensitivity is a feature of the other unstable hemoglobins is less clear. Administration of a sulfonamide did not shorten survival of erythrocytes containing Hb Sogn.<sup>50</sup>

Patients with Hb Köln disease usually have mild manifestations; however, this varies from one kindred to the next and even among different individuals in the same kindred.<sup>221</sup> Occasionally, moderately severe anemia has

been observed. Splenectomy usually was considered unnecessary, but, when performed, improvement generally occurred.

Hb Seattle was classified with the mild diseases even though the blood Hb level was about 10 g/dl. Signs of hemolysis were lacking (reticulocytes, 3%), and it was suggested that the low oxygen affinity of this variant made tissue oxygenation more efficient; thus, the red cell mass was "appropriately" decreased and erythropoiesis was not stimulated.<sup>244</sup>

A fourth group of hemoglobins are unstable *in vitro*, but are not associated with signs of hemolytic anemia on routine testing (Table 24-8).

Patients in any of the first three groups may complain of *dark urine*, varying from brown to almost black in color. In general, the degree of discoloration is related to the intensity of hemolysis. However, the patient may experience episodes of dark urine intermittently without clear relation to other signs of hemolysis. One review indicated that pigmenturia was found in 33 of 36 patients in whom search for it was made.<sup>227</sup> However, with some hemoglobins (eg, Philly, Riverdale-Bronx, Sydney, Sabine) no pigmenturia has been observed (see page 817). The pigment is thought to be a dipyrrole related to the mesobilifuscin group (Chapter 5) and to be derived from the degradation of the heme released when Heinz bodies form *in vivo*. Similar or identical pigments may occur in thalassemia (page 866).

### Laboratory Findings

The degrees of anemia and reticulocytosis and the occurrence of pigmenturia have been discussed above. The MCHC is reduced in many instances, sometimes to as low as 25 g/dl RBC. Presumably this occurs because the unstable molecule is partially heme deficient, because hemoglobin is lost from the cells with splenic removal of inclusions, or because the hemoglobin in Heinz bodies is not measured by hemoglobinometry. Microscopic examination of the blood usually reveals only nonspecific signs of hemolysis:

polychromatophilia, stippling, and anisopoikilocytosis; hypochromia may also be found. With severe disease, fragmented cells and microspherocytes may be observed. Moderate thrombocytopenia has been detected in a few patients, possibly because of splenic sequestration.<sup>231</sup>

*Inclusion bodies* (Heinz bodies) usually are found only after splenectomy or during an acute hemolytic episode. Under such circumstances, more than 50% of the cells typically contain one large, spherical inclusion when stained with methyl violet or brilliant cresyl blue. Schmid emphasized that these inclusions differ from the classic Heinz bodies seen with toxic exposures in that they are larger, are found in reticulocytes rather than older cells, and may be faintly visualized on Wright's stain.<sup>242</sup> When not present in fresh blood, inclusions may be induced to form *in vitro* by various techniques (page 809). The inclusions consist mainly of denatured globin, although various other cellular constituents, including porphyrins and nucleic acids, may be nonspecifically adsorbed. Usually, the precipitated globin lacks heme, but with some variants (eg, Hb Philly) the heme remains with the globin.

*Hemoglobin electrophoresis* may or may not detect the abnormality. Hemoglobin Köln migrates more slowly than Hb A when routine methods are used. Other variants may migrate slower or faster than Hb A, or may be inseparable from it. In a number of instances, an inhomogeneous streak trailing behind Hb A has been described, presumably the result of denaturation or heme loss occurring during electrophoresis. In such circumstances, a more discrete band may be seen if electrophoresis is performed at 10° C.<sup>227</sup> Electrophoretic behavior cannot be predicted from the change in charge induced by the amino acid substitution; the altered tertiary structure exposes some groups normally hidden and conceals others, thereby changing the overall surface charge. With  $\beta$ -chain unstable hemoglobins, free  $\alpha$ -chains may be found on electrophoresis; these typically have even slower anodal mobility than Hb A<sub>2</sub>.<sup>247</sup> In addition, heme-depleted unstable hemoglobin

( $\alpha\beta^{\text{heme}}\beta_2^*$ ) may be found, usually migrating just in front of Hb A<sub>2</sub>. Hb A<sub>2</sub> levels may be increased to as much as 5% in association with unstable  $\beta$ -chain variants, and Hb F levels may be increased to as much as 10 to 12%.

Certain abnormalities may be detected with *in vitro* hemolytic testing, but the findings are not specific. *Autohemolysis* is increased in a majority of patients<sup>227,247</sup> to values of about 5 to 6% in mild or moderate disease and as high as 15 to 25% in severe disease. There is partial correction with glucose and ATP; ie, a "Type I" pattern (page 735). Erythrocyte osmotic fragility may be normal, increased, or decreased.<sup>227</sup>

Various studies of *glycolysis* and the hexose-monophosphate shunt in erythrocytes have disclosed normal or increased overall rates, generally consistent with the immaturity of the cell population. Erythrocyte glutathione (GSH) levels may be high, normal, or low. When low levels are found, they probably result from binding of GSH by the unstable variant. GSH can form mixed disulfides with the normally reactive  $\beta 93$  cysteine and, as denaturation proceeds, with the normally unreactive  $\alpha 104$  and  $\beta 112$  cysteines.<sup>234</sup> Glutathione stability usually is normal, but may be slightly reduced.<sup>227,247</sup>

Erythrocyte survival often is shortened. Values for  $t_{1/2}$  Cr have varied from 2 days in severe disease, and 6 to 16 days in moderate disease, to 9 to 23 days in mild disease.<sup>235</sup> There is poor correlation, however, between the blood Hb level and  $t_{1/2}$  Cr,<sup>247</sup> and several factors may make the results misleading. For example, chromium binding and elution rates probably are abnormal in unstable hemoglobins.<sup>217</sup> DF<sup>32</sup>P studies probably would be more reliable, but have not been reported.

Because the nature of the amino acid substitution often affects heme function, the *oxygen affinity* of unstable hemoglobins may be disturbed. Oxygen affinity is *increased* (ie,  $P_{50}$  is decreased, page 107) in hemoglobins Köln, Shepherds' Bush, Casper, St. Etienne, Zurich, Freiburg, and Gun Hill. Oxygen affinity is *decreased* ( $P_{50}$  increased) with hemoglobins Torino, Leiden, Peterborough,

Seattle, Hammersmith, Bristol, and Louisville. In some instances, *methemoglobin* levels have been increased to about 5% in fresh blood.<sup>247</sup> More often, excessive methemoglobin (20 to 30%) has been found after sterile incubation at 37° C for 24 to 48 hrs.

## Diagnosis

At present the most reliable test for unstable hemoglobin disease is the heat denaturation test (page 808). This has been positive in all reported instances except for the patient with Hb Bryn Mawr, in whom the abnormal variant constituted only 2% of the hemoglobin.<sup>29</sup> With the quantitative heat denaturation procedure, 8 to 45% of the hemoglobin precipitates as compared with less than 1 to 2% in the normal. In Hb Köln disease, about 10 to 15% precipitates. With some variants, modifications of time and temperature have been required in order to demonstrate the heat lability.<sup>34</sup> Other tests for instability, including the isopropanol precipitation test (page 808) and precipitation with p-chloromercuribenzoate,<sup>234a,241</sup> may be equally reliable, but less information is available concerning them.

Precise identification of the abnormal hemoglobin requires peptide analysis (page 810), which is performed in only a few research laboratories throughout the world. However, for the clinical hematologist, exact knowledge of the substitution may not be necessary, since management does not depend upon it.

## Pathogenesis

The nature of the molecular lesions leading to hemoglobin instability were discussed on page 804. As a result of most of these lesions, water gains entrance to the heme crevice, making the heme iron susceptible to oxidation, and loosening the heme-globin linkage. When heme is lost, globin solubility is markedly reduced and usually unreactive cysteine sulfhydryl groups are exposed and form disulfide linkages with GSH and membrane sulfhydryl groups.<sup>234</sup> The heme-free

$\beta$ -chains precipitate, forming inclusion bodies, and the liberated heme is catabolized to dark-brown dipyrroles, which are excreted into the urine. Free  $\alpha$ -chains are left behind and may be detected during electrophoresis. With some variants (eg, Hb Philly and Hb Christchurch<sup>248</sup>) the heme probably remains with the globin after precipitation, and dipyrroluria does not occur. Formation of inclusions and their binding to membranes lead to loss of membrane sulfhydryl groups and increased cation permeability. Hemolysis occurs chiefly because cells containing inclusions are sequestered in the RE system, where either the inclusions are removed or the cells are destroyed, depending on the degree of damage.

## Treatment and Prognosis

Splenectomy may bring about some improvement in moderately affected patients.<sup>235</sup> Unfortunately, those with the most severe disease do not respond to the operation (Table 24-8), presumably because the more severely damaged erythrocytes are destroyed in the liver and other parts of the RE system as readily as in the spleen.<sup>247</sup> When splenectomy is to be performed, it is desirable to delay the operation until the patient is four years old or older, since the risk of infection appears to be increased in splenectomized younger children (Chapter 8).

Patients who are not candidates for splenectomy should be managed with the general measures recommended for hemolytic anemia (Chapter 20). Blood transfusions should be held to a minimum and usually reserved for a severe crisis. Sulfonamides and other drugs with a redox potential (Table 23-2, page 785) should probably be avoided, although, as already mentioned, their deleterious effects have been documented in only a few instances. Intercurrent infections should be detected and treatment instituted promptly. If there is reason to believe that the diet is inadequate, low doses of folic acid (0.15 to 0.3 mg per day) may be used prophylactically (Chapter 14).

The long-term prognosis remains un-

known in this relatively recently described illness. Only one death has been reported, probably from overwhelming sepsis in a splenectomized patient with Hb Hammer-smith.<sup>247</sup>

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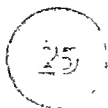


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## Hemoglobinopathies S, C, D, E, and O, and Associated Diseases

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### Introduction

In this chapter, sickle cell trait and sickle cell anemia as well as the more common hemoglobinopathies such as HbC, HbD, and HbE, as well as HbO, and their combinations with HbS will be considered. In Chapter 26, hemoglobinopathies associated with thalassemia are discussed.

All of the abnormal hemoglobins are in-

herited defects (Chapter 24). The spontaneous appearance of such mutations is exceedingly rare. Among patients who bear the hemoglobins to be described in this chapter the *heterozygote* rarely manifests any clinically significant phenotypic expression. In this sense these hemoglobinopathies are recessive traits. However, most heterozygotes are easily detected by simple laboratory tests. In that respect they are not phenotypically silent.

On the other hand, disease, often quite serious, is the usual consequence of the *inheritance of two genes* for these abnormal hemoglobins. This is true when the two genes are for the same abnormal hemoglobin, as in sickle cell anemia, or when two different genes are inherited, as in sickle cell-hemoglobin C disease.

### Terminology

A word about terminology will be helpful. When a person is found to have inherited one abnormal gene he is referred to as having the *trait* for that abnormality. If he has inherited two abnormal genes the disorder is referred to in several ways. The illness in persons who have inherited two HbS genes is called sickle cell anemia. In those who have inherited two different abnormal hemoglobins the designation incorporates the names of both, eg,

HbSC disease, HbS-thalassemia. Because its meaning is less clear than "sickle cell anemia," the term HbS disease is best avoided. The designation *HbS* or *sickle cell diseases* (SCD) is used, however, in a generic sense to refer to all the conditions in which the gene for HbS is associated with another abnormal hemoglobin gene, with accompanying clinical manifestations.

## Sickle Cell Trait and Sickle Cell Anemia

### Incidence and Geographic Distribution

As indicated in the preceding chapter, hemoglobin S is the result of the substitution of valine for glutamic acid at the sixth residue of the  $\beta$ -polypeptide chain of hemoglobin. The gene for HbS occurs with varying frequency in sub-Saharan Africa, the Mediterranean countries, and India and in the descendants of people who emigrated from these regions, namely, Negroes in South, Central, and North America. When inherited from only one parent, as mentioned above, HbS causes ill effects only under special circumstances (HbAS, sickle cell trait, page 839). Inherited from both parents, a chronic, severe hemolytic anemia (sickle cell anemia [SCA]) results.

There is much yet to be learned about the natural history of SCA. The symptoms vary considerably in severity from one patient to another, but they are essentially those attributable to the chronic severe anemia and to vascular occlusive episodes. The disorder is distinguished by the presence of peculiar poikilocytes that are commonly sickle or oat shaped. SCA has also been referred to as drepanocytic anemia and as meniscocytosis.<sup>62</sup>

Contrary to commonly held opinion, there is a high incidence of sickle cell anemia in tropical Africa. It has long been known as a symptomatic entity to health-care teams in the countries of this region.<sup>174</sup> Affected persons in Africa have at times been identified by limb-girdle tattoos and the disorder has been given onomatopoeic names denoting the character of the recurrent, unrelenting, pain-

ful, vaso-occlusive crises that the patient experiences.<sup>174</sup>

The HbS gene in Africa is geographically distributed in a broad, equatorial belt limited on the north by the Sahara Desert and the Ethiopian Highlands, on the south by two rivers, the Kunene and the Zambesi.<sup>230</sup> (Fig. 24-7, page 812.) Its relation to the endemicity of malaria was discussed earlier (page 813). The incidence of the sickle cell gene ranges from 0 in some mid-African tribes to 40% in others.<sup>192,354</sup> HbS is found in lesser frequencies in other parts of Africa. The average frequency in tropical Africa of HbS is 20%, and if one assumes that persons with SCA rarely reproduce, then an average frequency of SCA at birth would be about 10 per 1000 births. Considerable variation is found, however, within geographically adjacent regions. Thus, in Ghana a sickle trait incidence of 10% was found in the north and 20% in the south.<sup>174</sup>

In the Americas the gene frequency in Negroes is more uniform than in Africa because of the loss of African tribal identities and because the average American Negro has about 20% Caucasian ancestry.<sup>261</sup> Although the reported HbS gene frequency among Negroes in Latin America and the Caribbean varies, an approximation of 8% seems appropriate.<sup>230</sup> In the United States Negro the most likely frequency of the sickle cell trait also is 8% (see Table 24-7, page 813). The expected incidence of SCA at birth would be 1.6/1000 (one in 625). Without correction for mortality the expected number of cases of SCA among the approximately 20 million Negroes in the United States would be about 32,000. Because of the death rate, it has been estimated that the actual number is about one half to two thirds of this figure.

There is a substantial incidence of simultaneous heterozygosity for the HbS gene and one of the genes responsible for thalassemia or one of the other hemoglobinopathies such as HbC, HbD, or HbE. In Ghana the 10 to 20% incidence of HbC trait and the 10 to 20% incidence of HbS trait mean that about 1% of the children have HbSC disease.<sup>174</sup> Thus, in Ghana alone, with a population of

8 million, 30,000 of each million newborns will have either HbSS, HbSC, or HbCC disease.

If one considers HbSC disease and HbS-beta-thalassemia together in the Negro population of the United States, there should be almost as many or even more newborns with these disorders than with sickle cell anemia alone (36,000).<sup>221</sup> (Table 24-7, page 813.) Since the mortality rate of these two disorders is less than that of SCA, their incidence in the population should exceed that of SCA. Similar estimates of the frequency of HbSC disease and possibly of HbS-thalassemia in Central and South America are probably warranted.

### Pathogenesis: Molecular and Cellular Pathology

#### The Sickling Phenomenon

Early studies demonstrated that the erythrocytes from patients with sickle cell disease (SCD) assume the sickle shape when the hemoglobin that they contain is deprived of oxygen.<sup>93,117,121,126</sup> In addition, sickling was shown to be favored by a lowering of pH<sup>303,325</sup> and by increasing the temperature to that of the body. The first visible cellular change, usually detectable within 10 seconds after deoxygenation, is loss of the red cell flicker phenomenon, a periodic movement within the red cells visible by phase microscopy. Simultaneously, or shortly thereafter, a mass of hemoglobin appears to flow to one side of the cell. The cell becomes ovoid, elongated, and crescent-shaped, and ribs of solidified hemoglobin extend from the hemoglobin mass to join a rim of hemoglobin at the opposite perimeter of the cell (Fig. 25-1).<sup>122,236,251</sup> Prolonged deoxygenation results in increased deformation of the cell, and long, thin filaments extend from the body of the cell. These filaments are easily broken off when the sickled cell is manipulated.<sup>154,158</sup>

Reversion to a normal disc shape (unsickling) occurs upon oxygenation of the cell. The changes probably begin within 10 seconds after oxygen reaches the cell and first

become apparent in the projecting filaments. The liquefied hemoglobin may flow from these spicules toward the body of the cell or may become trapped and form a membrane-enclosed hemoglobin bead. Such beads may become detached from the cell during the unsickling process. As the intracellular hemoglobin liquefies, it tends to flow first along the internal rim of the cell and finally into the central portion. At that time the red cell flicker phenomenon reappears.<sup>236</sup>

After being repeatedly subjected to sickling and unsickling, the cell may lose the ability to revert to the normal biconcave discoid shape.<sup>304</sup> Such "irreversibly sickled cells" (ISC's) probably result from the loss of portions of membrane as the microfilaments are shed.<sup>236</sup> Repeated sickling may also cause the formation of inclusions with the morphologic characteristics of small Heinz bodies. These inclusions become attached to the membrane and are partially responsible for premature destruction of the cell.<sup>183,209,284</sup>

#### Molecular Mechanisms

An abundance of information indicates that the alterations in the shape of cells containing HbS are the result of hemoglobin polymerization.<sup>126,245</sup> Cell-free solutions of HbS undergo a pronounced decrease in solubility and an increase in viscosity when deoxygenated.<sup>8,29,30,37,125</sup> If the concentration of HbS in such solutions or in the red cell approaches 30 g/dl, a semisolid gel forms, and the gel contains small, rigid, boat-shaped objects that polarize light. These objects are oematic liquid crystals known as tactoids.<sup>31,126,259,305</sup>

By electron microscopy the polymers of HbS are found to be long, rod-like structures. Each rod is formed from six monomolecular strands of hemoglobin S twisted into a spiral. Tactoids are formed from organization of the rods into bundles. Within the sickled cell these linear polymers lie parallel to the long axis of the cell or projecting filaments.<sup>80,135,186,259,321,343,349</sup>

Although the nature of the molecular interactions producing polymerization is not completely understood, it may be assumed

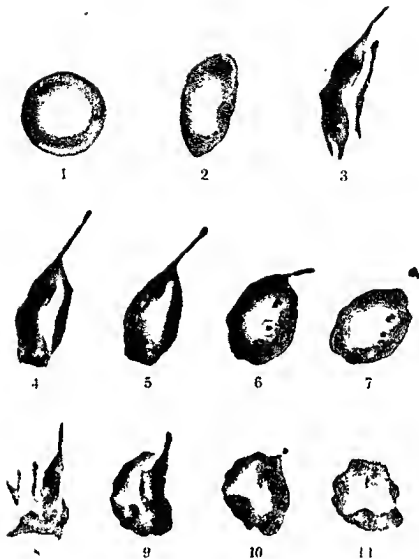


Fig 25-1. Photographs of a single cell, suspended in plasma (1), which on deoxygenation became ovoid (2) then sickled with spicules and spikes (3), which on oxygenation retracted towards the body of the cell (4-5). The long spicules became pinched at the cell junction (6) and hemoglobin enclosed in membrane formed a fragment which separated (7). A second deoxygenation produced the changes seen in (8) and (9) and reoxygenation (10) ultimately resulted in the permanently irregular "lumpy-bumpy" cell (acanthocyte) shown in (11). All of the protrusions or knobs are permanent deformities, and are located where spikes or spicules were formed during the deoxygenation of the cell.

that the substitution of valine for glutamic acid at  $\beta^6$  creates a site which, when the molecule is deoxygenated, is capable of forming a bond with a normal site on an adjacent molecule.<sup>58,223,224,247</sup> The complementary site is present even on normal or non-HbS abnormal hemoglobin molecules,

whether they be in the oxy- or deoxygenated state.

The observations that decreased temperature, high oxygen pressures, and the carbamylation of the N-terminals of HbS inhibit gelation support the hypothesis that hydrophobic intramolecular bonds form between

the  $\beta$ -N-terminal valine and the abnormal valine at  $\beta^6$  to form a ring. It has been suggested that formation of the ring creates this abnormal site that is involved in polymer formation.<sup>221</sup> However, the observation that salt-depleted hemoglobin solutions gel at lower concentration than do salt-replete solutions suggests that electrostatic bonds also participate in the polymerization.

In forming polymers, HbS reacts to a variable extent with other hemoglobins. In the test tube, a deoxy-HbS molecule copolymerizes most effectively with another HbS molecule, and, in decreasing order, with hemoglobins C, D, E, A, J, and F.<sup>6,24,30,54,708</sup> These observations correlate with the severity of the clinical manifestations of disorders in persons in whom various combinations of these hemoglobins are found. Thus, patients with sickle cell anemia, whose cells contain more than 90% HbS, have the most serious disease, followed by those with sickle cell-HbC disease. In contrast, individuals doubly heterozygous for HbS and hereditary persistence of HbF, whose cells contain about 70% HbS and 30% HbF, rarely if ever have clinical disease.<sup>39,68,218,200</sup>

Two other sites on the hemoglobin molecule have been implicated in the interaction because of the effects of substitution at these sites. In Hb Memphis, the substitution  $\alpha 23$  (B4) Glu  $\rightarrow$  Gln inhibits copolymerization with HbS, suggesting the importance of sites on the  $\alpha$ -chain.<sup>132,179</sup> On the other hand, the substitution of  $\beta 73$  (E17) Asp  $\rightarrow$  Asn in HbC Harlem and HbS Korle Bu also inhibits polymerization.<sup>37,38,176</sup>

### Mechanisms of Hemolysis

It has been estimated that about one third of the total cell destruction in SCA takes place intravascularly.<sup>21,72</sup> Such intravascular hemolysis probably represents the shedding of microfilaments and progressive cellular fragmentation as the relatively rigid sickled cell passes through the microcirculation (page 824).

Extravascular hemolysis accounts for the

remaining two thirds of the destructive process.<sup>24</sup> Cells containing small Heinz-like bodies (page 824) are susceptible to phagocytosis by macrophages in the spleen and other RE organs.<sup>188,280</sup> In addition, a number of important biochemical and physical alterations adversely affect the survival of irreversibly sickled cells. As compared with other cells in the circulation of patients with SCD, ISC's are small cells with higher HbS concentrations, lower HbF concentrations, increased density,<sup>61</sup> decreased deformability, reduced membrane lipids,<sup>154</sup> normal 2,3DPG levels, reduced  $O_2$  affinity,<sup>293</sup> and increased content of calcium<sup>58a</sup> and increased cationic flux.<sup>331</sup> These features are similar to those described in preterminal, senescent normal cells (Chapter 5, page 201). Presumably, one or more of these changes stimulates phagocytosis by a reticuloendothelial macrophage.

### Vascular Occlusive Disease

Deoxygenation of blood from patients with sickle cell disorders brings about a pronounced increase in whole blood viscosity, which is due to the net effect of an increase in the internal viscosity of each cell.<sup>61</sup> In addition, sickled cells, being relatively rigid, are unable to alter their shape to the degree necessary to flow through capillaries 4  $\mu$ m in diameter or smaller (see Chapter 3, page 92). These two factors lead to dramatic reductions in blood flow within certain capillary beds when oxygen levels drop. The stagnation that follows leads to further reductions in oxygen tension and additional sickling, thereby aggravating the situation.<sup>129,157</sup>

Vascular occlusions in sickle cell disease typically occur in the microcirculation, particularly in vascular beds that are characterized by slow flow and high oxygen extraction. There appears to be a correlation between the frequency and severity of involvement of different organs and their circulatory characteristics. One frequently affected organ is the heart; because the myocardium extracts oxygen more efficiently than any other organ, there is almost always clini-

cal and pathologic evidence of thrombosis, infarction, and fibrosis in that organ as the result of vascular occlusion as well as the long-term result of anemia and iron overload.<sup>191</sup> In addition, the spleen, bone marrow, and placenta all are characterized by low blood pressure, slow blood flow, and high oxygen extraction, and all commonly participate in vascular occlusive episodes leading to infarction and fibrosis.<sup>81</sup> The effects of vascular occlusion in other tissues will be discussed below.

### Susceptibility to Infection

Patients with SCD have increased susceptibility to infection with certain bacteria and other infectious agents.<sup>20,95,307</sup> The most commonly involved sites are the lungs,<sup>21,162,295</sup> the genitourinary tract, and the bones and joints.<sup>70,139</sup> Less often the skin and the central nervous system are persistent sites of infection. There are several possible reasons for the increased propensity to infection: (1) When infection follows vascular occlusion, access of leukocytes to the involved area may be impeded. (2) Phagocytic function of the reticuloendothelial system may be impaired in patients with SCD, particularly in children with functional "hypoplasia."<sup>243</sup> (3) There is evidence for deficient opsonin activity in some children with SCD; possibly this abnormality represents a defect in the properdin system, which normally provides an important pathway for activation and fixation of the C3 component of complement to the surface of such bacteria as pneumococci. A decrease in this activity impedes ingestion of pneumococci by phagocytic cells.<sup>159,270,351</sup> (4) Defective granulation<sup>35</sup> and decreased phagocytic capability of the polymorphonuclear leukocytes have been described,<sup>85</sup> but the latter observation needs confirmation.

Alteration of one or more of these mechanisms and hypersusceptibility to infections seem to be more common in childhood than in adulthood, but in patients of all ages the differentiation between infarction, infection,

or the presence of both provides a difficult diagnostic problem.

### Clinical Characteristics of Sickle Cell Anemia (SCA)

#### Onset

Symptoms of SCA usually first become evident during the second half of the first year of life.<sup>240</sup> The lack of symptoms during intrauterine life and in the immediate postnatal period is accounted for by the presence of larger amounts of fetal hemoglobin than are present later. As the adult pattern of hemoglobin becomes established, and the proportion of abnormal  $\beta$ -chains increases, the individual becomes more and more susceptible to the pathophysiologic events that cause clinical symptoms. By about six months of age, the child's cells have acquired the adult complement of HbS. There is, however, some variation in the age of onset of symptoms; this ranged in one series from three months to 15 years.<sup>120</sup>

Just as the age of onset of symptoms varies, so does the severity of the disease. The usual presentation is that of a patient with severe hemolytic anemia, painful vaso-occlusive episodes, and multiple organ involvement. Much less often, an individual who inherits HbSS has only moderate anemia, few symptoms, and a nearly normal life span. Some patients with what appears to be mild sickle cell anemia may in fact be doubly heterozygous for combinations of HbS and  $\beta$ - or  $\alpha$ -thalassemia, or have HbSD disease, or HbS associated with hereditary persistence of fetal hemoglobin (page 875).<sup>275</sup> In other instances the mild nature of the disease remains unexplained. In a group of patients without evidence for double heterozygosity, attempts to relate the severity of symptoms to various laboratory findings were without success. Neither the hemoglobin concentration, reticulocyte count, nor the HbA<sub>2</sub> or fetal hemoglobin levels could distinguish patients with relatively benign manifestations from those with more severe SCA.<sup>320</sup>



In children, the onset of SCA is often heralded by irritability and complaints of pain in joints, the back, the legs, the abdomen, or chest. In addition to such painful episodes, children manifest other characteristic clinical syndromes. A most distinctive one is dactylitis (*the hand-foot syndrome*). This, in about one third of the patients, is the first serious symptomatic episode. Typically, the dorsa of the hands and/or feet are swollen, non-erythematous, and exquisitely painful. Fever and leukocytosis are common and the more severe symptoms last for 10 to 14 days. Roentgenologic changes, usually detectable after about a week, consist of periostitis and osteolysis in addition to the soft-tissue swelling. The episodes are not altered by specific medication. They are self-limited and rarely occur after the patient is three years of age.<sup>219,339</sup>

As they grow older, the patients become susceptible to the symptoms that attend involvement of various organs; the painful crises that typify sickle cell anemia in adolescent and adult patients make their appearance.

### Growth, Development, and Life Span

The growth pattern of children with SCA differs from that of normal children. The afflicted children are thinner than age-matched normal children; growth is retarded in 94% of them<sup>298,350</sup> and their mean weight is 2 SD below the mean weight of normal children. Sexual and skeletal maturation may be delayed. Adult patients often are underweight and have relatively long extremities, narrow hips, and an accentuated lordotic curvature. However, exceptions to the typical SCA habitus are common enough to suggest that the classic descriptions of the thin, asthenic, long-limbed, short-trunked individual have overemphasized this feature.

Changes in the skeleton may occur as the result of bone marrow hyperplasia, the expansion of medullary spaces leading to prominence of the frontal bones or to maxillary bone overgrowth (gnathism).<sup>174</sup> Such abnormalities probably are less striking today

than they were a decade ago, possibly because of improved nutrition, decrease in the number of infections, and better management of parasitic infestations.<sup>174,311</sup>

Adequate data concerning the life span of patients with sickle cell disease are not available for large populations in Africa<sup>174,230</sup> or in the Americas, but one study indicated that more than 50% of patients with SCA are older than 10 years of age and thus it is apparent that the prognosis at birth is not entirely hopeless.<sup>300</sup> Although completely satisfactory data for average survival are not available, it appears that most patients do not survive beyond the second and few survive beyond the fourth decade of life. In one study the median survival was found to be 14.3 years; approximately one fifth of the deaths occurred in the first two years of life, one third before the fifth year, one half in patients between ages 5 and 30 years, and one sixth in those older than 30.<sup>81</sup> SCA has been reported, however, in patients of relatively advanced age,<sup>1,56</sup> and, in Jamaica, 121 patients over 30, 43 over 40, and 13 over 50 years of age were observed.<sup>300</sup>

### Sickle Cell Crises

The term "sickle cell crisis"<sup>79</sup> may have been used too freely in discussions of the clinical aspects of SCA, and this practice has sometimes tended to blunt the physician's search for an intercurrent disease, unrelated to the sickle cell disorder. The phrase "sickle cell crisis" is probably most appropriate when used to refer to the uncomplicated, painful vaso-occlusive episodes. A typical crisis is best described as an episode of moderate to severe pain, resulting from occlusion of portions of the microcirculation. Such episodes are characteristic of the sickle cell disorders and occur only rarely in association with other hemolytic disorders. The pain, which is gnawing, gradually increases in severity over a period of hours and commonly involves the extremities, especially the tibial and periarticular areas, the abdomen, chest, and back.<sup>53,79,174</sup> Once the symptoms have appeared, they are unlikely to change in

character. They may last only a few hours but, more often, they persist for a few days or weeks. The pain is periodic and usually abates gradually. The episode may be preceded by an infection. Fever, usually of low grade and not associated with chills, may occur. There tend to be no specific physical or laboratory findings that identify the episode as a painful crisis; rather, the very absence of such specific findings is important in the recognition of a vaso-occlusive painful crisis.<sup>15</sup> The diagnosis is greatly facilitated by prior experience with the patient since many have a stereotyped pattern of pain that is recognized by both patient and physician.

The diagnosis requires particularly careful clinical evaluation because patients with SCA are more than normally susceptible to other painful disorders. Thus the painful crisis must be differentiated from pneumonia, pulmonary infarction, gout, acute rheumatic fever, rheumatoid arthritis, acute pyelonephritis, cholecystitis, and a variety of other conditions.

### Cardiovascular Manifestations

Cardiovascular abnormalities are constant features of SCA and the other symptomatic sickle cell disorders. The heart is subjected to repeated episodes of acute and chronic stress, and the extent and type of vascular disease seem unique to the sickle cell disorders as compared with most other types of chronic hemolytic anemia.<sup>88,170,191</sup> Early in childhood, patients with SCA develop cardiac enlargement, and murmurs are almost always present. Common cardiorespiratory symptoms include exertional dyspnea, palpitation, and pleuritis. Rarely the patients experience pain that suggests myocardial ischemia. All of the features of a hyperdynamic circulation may be observed. The arterial pulses are full and brisk, reflecting a widened pulse pressure. The jugular venous pulse usually is normal, but occasionally it is characterized by an exaggerated A wave. The precordium usually is hyperactive, a manifestation of biventricular hypertrophy. The apical impulse may be

enlarged, sustained, and displaced to the left and an anterior parasternal lift is common.

Systolic murmurs can almost always be detected. These tend to be quite loud and usually are located near the left sternal border with occasional radiation to the entire precordium. Often pansystolic, they may mimic the murmur of mitral regurgitation and may be associated with an ejection click. The systolic impulse in the second left intercostal space is often visible and sometimes palpable, a finding that represents the transmission of pulmonary artery pulsations to the chest wall. Although these findings suggest pulmonary hypertension, the pulmonary artery pressure usually is normal.<sup>306,317,332,334</sup> The auscultatory events reflect the rapid ventricular filling. A loud third heart sound is almost invariably heard and the fourth heart sound often is also detected.

Radiologic examination reveals cardiac enlargement of moderate or severe degree involving all chambers, and the silhouette is somewhat globular (Fig. 25-2). The pulmonary artery segment of the border of the left side of the heart frequently is conspicuous, and other pulmonary vascular shadows are prominent. The right and left main branches of the pulmonary arteries may be markedly enlarged.

The electrocardiogram commonly shows abnormality,<sup>334</sup> but without specific diagnostic findings. Electrocardiographic evidence of left ventricular hypertrophy is obtained in about one half of the adult patients and signs of right ventricular overload are found in about 10 to 15%. The multiplicity of cardiovascular abnormalities attributable to SCA makes the differentiation from such disorders as rheumatic fever, congenital heart disease, and bacterial endocarditis particularly difficult. A number of reports document the concurrence of SCA and rheumatic heart disease, atrial septal defects, pulmonary stenosis, or tetralogy of Fallot,<sup>170,202,208,232</sup> but there is no reason to believe that these conditions are more frequent in SCA than in the general population. They are best differentiated by cardiac hemodynamic studies or by angiography.

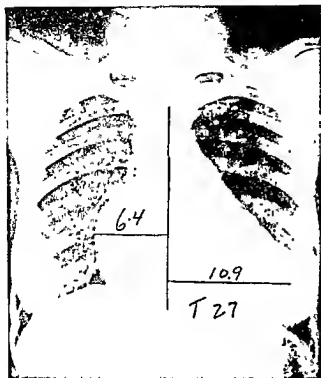


Fig 25-2. Teleoroentgenogram of the heart and great vessels in a patient with SCA. The heart is markedly enlarged with prominent left ventricle and right atrium. There is a slight prominence in the region of the pulmonary conus.

### Pulmonary Signs

Since the sickling phenomenon depends on deoxygenation of hemoglobin, adequate pulmonary function and oxygenation are essential to the well-being of the patient with SCA.<sup>42,214</sup> The major acute pulmonary disorders in these patients are *infectious* and *vascular occlusive episodes*. These may occur separately or in combination and may be extremely difficult to distinguish from each other. Indeed, they may be pathogenetically interrelated and concurrent.<sup>21</sup> *Chronic pulmonary disease* may result from repetitive episodes of infection and from vascular occlusion leading sometimes to chronic pulmonary arterial hypertension and cor pulmonale. The most common pulmonary functional abnormality is decreased arterial oxygen tension and desaturation of arterial blood.<sup>42,43,44,157,317</sup>

Of 164 patients with SCA and evidence of bacterial pneumonia, pneumococcus was recovered from sputum or nasopharyngeal cultures in 67, and from the blood in 11. Other causative organisms included *Mycoplasma*

pneumoniae, *Haemophilus*, the *Salmonellae*, *Escherichia coli*, and, less frequently, other gram-negative rods and gram-positive cocci.<sup>19</sup> In patients with SCA, bacterial and mycoplasmal pneumonias last longer than in normal subjects; furthermore, infiltrates commonly affect more than one lobe of the lungs, and resolution of the infiltrates is slower.<sup>19,249,307</sup> It is likely that impaired access of oxygen to the infected lung tissues enhances the sickling phenomenon with resulting local, microvascular thrombotic disease. The bacterial pneumonias may be associated with bacteremia and metastatic infections, and, therefore, with disseminated intravascular coagulation.<sup>162,264</sup> They require prompt identification and energetic antimicrobial therapy. The aggressive use of diagnostic techniques, including transtracheal or transthoracic needle aspiration of bronchial material or lung tissue as well as bronchial brushings, is warranted to establish a specific diagnosis and to allow specific therapy.

Pulmonary vascular occlusion occurs commonly in the absence of infection, and sudden

death following large vascular occlusion has been reported.<sup>131</sup> Patients with SCA develop *in situ* vaso-occlusive disorders produced by the sickling of relatively deoxygenated blood in the pulmonary arteries; embolization to the lungs of clots formed in distal veins also may occur.<sup>44</sup> In addition, bone marrow and fat emboli from infarcted marrow are known to have produced vascular pulmonary disease.<sup>81,233,316</sup> The differentiation of primary infection from vascular occlusion is difficult. Clinical features that favor a diagnosis of pulmonary thrombosis are associated pain in the abdomen or extremities, absent or minimal temperature elevation, a lack of chills, inability to identify an offending organism, and perhaps the presence of poikilocytes ("blister cells") and an increased number of fragmented cells (ISC's) in the blood.<sup>16,21,165</sup> Pulmonary radioisotopic scans so far have not proved to be very useful in differentiating thrombosis from infection. Experience is inadequate to allow evaluation of pulmonary angiography.

Pulmonary mechanical function usually is impaired at least modestly and sometimes strikingly in patients with SCD. Typically, there is a modest reduction in vital capacity and total lung capacity.<sup>44,96,212</sup> Occasionally there is obstructive lung disease, and gas mixing and gas exchange almost always are abnormal. Arterial oxygen tension ranges from 70 to 90 mm Hg, accompanied by a reduction of arterial saturation usually ranging from 80 to 90%, but occasionally falling to even lower levels.<sup>44,317</sup> Blood may be shunted through an undefined pathway or there may be disparity between ventilation and perfusion. The combination of these various processes results in a decrease in the available cross-sectional, pulmonary vascular tree and, at times, an increase in pulmonary artery pressure. In some patients the pulmonary hypertension contributes to the cardiac disease.

#### Abdominal Involvement

Episodic abdominal pain is common in patients with SCA and is a frequent feature of the painful vaso-occlusive crises (page

828). The organs most often involved include the liver, gallbladder, and spleen, whereas the stomach and intestines seem to be relatively infrequent sites of symptomatic disease.

The *liver* usually is enlarged, but is not tender to the touch. However, it may enlarge acutely and become quite tender during periods of crisis. Subcapsular infarcts may occur and these may produce right upper quadrant pain and transient rubs. Liver abscesses have been reported, apparently the result of bacterial inoculation of infarcted tissue.<sup>40</sup>

In older patients, a diffuse or nodular type of cirrhosis may be found.<sup>36,115</sup> Various forms of intercurrent acute liver disease occur with increased frequency in patients with SCA. These include viral hepatitis,<sup>17</sup> drug-induced liver disease, hemosiderosis, and the complications of cholelithiasis.<sup>115,271</sup>

About 30 to 60% of patients, depending on age, develop gallstones but not more than 10 to 15% have symptoms that may be attributed to disease of the biliary tract.<sup>18,48,231</sup> The gallstones usually are radiolucent, and the gallbladder is ordinarily visualized by radiopaque dye studies, despite the intermittent hyperbilirubinemia.<sup>123</sup> The clinical manifestations of cholecystitis are similar to those in patients with cholecystitis who do not have SCA. Because of the high incidence of cholecystitis in patients with SCD and difficulty in the differential diagnosis of right upper quadrant pain in these subjects, some have suggested elective cholecystectomy for SCD patients with cholelithiasis.<sup>18</sup> The expected operative morbidity and mortality are probably not greater than those of age-matched patients who do not have SCD.

The *spleen* and *splenic function* almost always are altered in SCA. Splenic function may be increased, or decreased, and may change rapidly, or, in some patients, over long periods. Most commonly in childhood, the spleen is enlarged without evidence of hyperfunction or hypofunction. Occasionally, in childhood, the *splenic sequestration syndrome*<sup>45</sup> occurs. The spleen undergoes rapid enlargement from the acute entrapment of red cells, with resulting systemic hypovolemia, severe abdominal pain, more severe ane-

mia, and other findings indicative of an abdominal catastrophe.<sup>151,210,291</sup> "Hyposplenism" may be suspected when the spleen is *not palpable*, or even when it is palpably enlarged but pneumococcal infection occurs, or when there is thrombocytosis or marked leukocytosis and target cells and increased numbers of nucleated red cells and Howell-Jolly bodies are found in the blood.<sup>210,212,213</sup> By adolescence, the spleen is enlarged in fewer than 15% of SCA patients in the United States.<sup>210</sup> When splenomegaly is detected in late adolescence or adulthood, the patient should be studied for the possibility of doubly heterozygous disease (pages 844, 872) or the concurrence of another illness. In adult patients, the spleen usually has undergone repeated episodes of thrombosis, infarction, and, occasionally, military calcification ("autosplenectomy").<sup>78,134,316</sup> Occasionally in SCA, but more commonly in the other sickle cell disorders and in sickle cell trait, acute splenic infarction is precipitated by exposure to high altitude.<sup>144,310</sup>

### Bone and Joint Changes

The dactylitis seen in infants and young children with SCD (page 828) is one of the many changes that occur in the bones and joints of most patients with SCD. The slow sinusoidal circulation of the bone marrow provides an ideal vascular bed for the sickling phenomenon and *bone marrow infarction is a common event. Evidence that bone marrow circulatory occlusion occurs in SCA includes the frequency of bone pain during painful crises, the occurrence of bone marrow embolization, the radiologic changes in the bones, and the abnormalities found on histologic examination of bone marrow biopsy specimens as well as at autopsy.*<sup>58,81,316</sup> The vascular occlusions cause avascular necrosis of the hip or shoulder, which may mimic the clinical and radiologic appearance of Legg-Calvé-Perthes disease.<sup>63</sup>

Bone disease in SCD often is associated with degenerative changes of cartilage and synovial membranes, which cause deformities of adjacent joints. Marrow infarction, necrosis, and the healing process are often followed

by new bone formation which produces unique radiologic abnormalities (Fig. 25-3). When larger segments of cortical bone undergo infarction, periosteal reaction and subperiosteal calcification follow. The bodies of the vertebrae contain hyperplastic marrow and the distorted trabecular structure may be inadequate for normal weight-bearing. In adults the vertebral bodies may become compressed and also develop concave central regions, a deformity known as "fish-mouth vertebrae."<sup>80</sup> Marrow hypertrophy in the flat bones causes widening of the diploic space; this produces the thickening of the frontal bones and the maxillary bone overgrowth mentioned earlier (page 828).<sup>174</sup> In Africa, *osteomyelitis* is much more common in patients with SCA than in persons who do not have this disease.<sup>174</sup> There, and also in the United States, the most common infecting agents are the various species of salmonellae. *Staphylococcus aureus* also is a common causative organism.<sup>80,91,139</sup>

The joints are affected by a number of disorders including avascular necrosis of bone adjacent to a joint, gout,<sup>13</sup> septic arthritis, possibly hemarthrosis and hemosiderosis, and a peculiar arthritis in which the knees and elbows are commonly involved. The arthritic episodes are painful and often are associated with fever, leukocytosis, and joint transudates. Synovial biopsies show obliterated vessels without evidence of acute inflammation or marrow embolization. Sickled erythrocytes are found in the joint fluid.<sup>287</sup> In adults, there may be deformations of the hands and feet, with shortening of digits, apparently the remote sequelae of the dactylitis of childhood.<sup>298</sup>

### Genitourinary Signs

SCA is associated with a variety of defects in renal function.<sup>46,281</sup> In most patients with SCA and in some with sickle cell trait, the ability to concentrate urine<sup>167,244</sup> and to excrete hydrogen ion<sup>113,129,140</sup> is impaired. These abnormalities usually are not severe and produce no symptoms. The concentrating defect seems to be related to the age of the individual and the severity of the dis-



Fig 25-3 Sickle cell anemia. A, Femur. The cortex is thinned and the normal bony architecture is disturbed. Adjoining small areas of translucency there are areas of sclerosis. B, Tibia and fibula. Marked thinning of the cortex of the bones as well as periosteal reaction and disarrangement of the trabeculae. The latter changes and the extensive coarseness of the cortical layers suggest that the bone is involved from within.

ease. It is reversible in young but not in older persons by the transfusion of normal blood.<sup>167,318</sup> For this reason, it has been suggested that, in early life, blood flow in the minute vessels of the renal medulla is impaired by the presence of sickled cells, and, later, a portion of the vasa recta is obliterated.<sup>168</sup>

Hematuria is common, and may be brisk and prolonged. The hemorrhages may come from one kidney or both or occur in alternating fashion. The bleeding possibly results

from congestion and thrombosis of the renal mucosal vessels or, less often, comes from the papillae.<sup>6,112,127,220</sup> The rapid red cell turnover in sickle cell disease is attended occasionally by hyperuricemia, which, in some instances, may cause attacks of gout and, less commonly, urate nephropathy.<sup>109,337</sup>

The nephrotic syndrome occurs only rarely, but less severe vascular and glomerular abnormalities are considerably more frequent. Glomerular basement membrane changes have been detected in young pa-

tients,<sup>252</sup> and similar but more advanced lesions were found in three patients with the nephrotic syndrome.<sup>203</sup> It is possible that the nephrotic syndrome is caused by a combination of vascular thrombotic events and the deposition of non-heme iron in the glomerular epithelium and the proximal tubular epithelium.<sup>10,252</sup> Alternatively, it may result from renal venous thrombosis or from unrelated disease.

Ultimately the majority of patients with SCA develop moderate or severe renal functional impairment, but end-stage renal failure is an unusual cause of death.<sup>328,337</sup>

*Priapism* of a severe degree is uncommon, but many patients relate that they have had transient episodes. The engorgement of the corpora cavernosa usually is not accompanied by dilatation of the glans penis. There are no recognized precipitating causes. The episodes last for hours or for days.<sup>105,165,373</sup> Numerous irreversibly sickled cells and clots have been found in the distended sinuses when therapeutic needle aspiration or incisional drainage has been carried out.<sup>81</sup> The repetitive trapping of cells in the corpora cavernosa, with or without surgical intervention, may lead to fibrosis of the septa and irreversible damage to the arteriovenous mechanisms of penile erection.<sup>314</sup>

## Neurologic Manifestations

Infections occur in the central nervous system, but most of the neurologic damage results from vascular disease. Although it has been widely held that the neurologic abnormalities are caused by occlusions of venous capillaries and precapillary arterioles, both cerebral angiography and pathologic studies have demonstrated thrombotic involvement of medium and large vessels. In an angiographic study, partial or complete occlusion

of large cerebral vessels was found in six of seven patients with SCA who manifested central nervous system dysfunction.<sup>322</sup>

These angiographic findings are in keeping with the high incidence of central nervous system abnormalities in patients with SCA. Thus, in a study of 89 patients observed for five years, hemiplegia, usually in conjunction with other neurologic symptoms, occurred in 17%; convulsions were noted in 12%, disorders of consciousness in 9%, and visual disturbances in 5%. Intracranial hemorrhages and spinal cord infarction also have been observed.<sup>255</sup> Diagnostic evaluation may require not only routine radiologic procedures and cerebrospinal fluid examination, but also radioisotopic brain scans and cerebral angiography. Angiographic contrast media have the potential for initiating sickling and careful attention must be given to proper preparation of the patient and to the rate at which dye is introduced into the artery.<sup>39</sup>

Peripheral neuropathy is no more common in SCA patients than in the normal population, and intellectual capacity and ability for mentation and scholastic activities seem unaffected except when specific cerebrovascular neurologic damage has occurred.

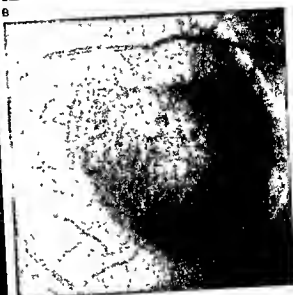
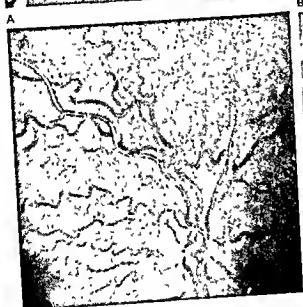
## Ocular Manifestations

The ocular manifestations of SCA are due either directly to involvement of the vasculature or are the sequelae of vascular damage.<sup>11,66,69,190,343</sup> The conjunctival vessel changes, most often seen in the lower temporal bulbar conjunctivae, consist of dilated segments of capillaries or veins that are packed with sickled cells and have a comma-shaped or curlicued appearance (Plate XII, A).<sup>58,237</sup> These lesions are best seen with the slit-lamp biomicroscope and are rare in disorders other than SCA, HbSC disease,

## PLATE XII

*Common ocular abnormalities in sickle cell anemia* The "comma" vascular sign shown in A is a superficial conjunctival vessel that contains densely packed sickled cells. B shows the interrupted or segmental flow of cells characteristic of sludged blood in a superficial conjunctival vessel. C shows the widened veins and tortuous large vessels of the retina. In D is seen a large preretinal hemorrhage, of approximately two weeks' duration, which has undergone partial resorption and has exposed a darkened area that was the probable site of intraretinal hemorrhage. E shows a stained blood smear of a patient with sickle cell anemia. In F an old pigmented choroidal scar is shown. (Photographs by Professor Mansour Amaly, The George Washington University Medical Center.)

# PLATE XII



E

F



HbSD disease, and HbS-thalassemia.<sup>65,111</sup>

Repeated *vitreous hemorrhages* may arise from retinal vascular anomalies, from areas of neovascularization, or from acute occlusion of large veins. The blood may be gradually resorbed, or become organized to form fibrovascular membranes with attachments at the base to the retina.<sup>66</sup> Contracture of these membranes may cause giant retinal tears or retinal detachment with recurrent hemorrhages into the vitreous. Obstruction of retinal vessels may lead to hemorrhage or to thrombosis followed by the formation of diffuse patchy areas of devascularization. Retinal vessels may be tortuous and looping. These "horseshoe"-shaped veins, the microaneurysms, and the heavily pigmented areas of prior chorioretinal infarction ("sunburst" lesions) are typical fundusoscopic findings in the sickle cell disorders (Plate XII). When retinal arteriolar occlusions occur, arteriovenous anastomoses may form, followed by a quite typical process of neovascularization that produces vessels resembling "sea fans,"<sup>110,111</sup> lesions which are much more common in HbSC disease than in SCA.<sup>110</sup>

### Pregnancy and HbS

Pregnancy and SCA affect one another adversely. The highest morbidity and mortality were recorded in earlier reports,<sup>73,92,268</sup> the more recent observations suggesting much lower rates.<sup>141,245</sup> Complications in the first six months of pregnancy are infrequent except that anemia may become more severe, and there may be an increased incidence of pyelonephritis, hematuria, and painful crises.<sup>141,245</sup> During the latter months of pregnancy and throughout parturition and the first few postpartum days, the patient is at greatest risk. Mortality rates ranging from 0 to 25% have been reported. Heart failure, which occurs in 2 to 20% of SCA patients, may appear for the first time during pregnancy; about 10% of pregnant SCA patients develop phlebitis, and pulmonary infection and/or infarction occur in 5 to 40%. Toxemia is common and postpartum puerperal endometritis occurs in about 20% of these subjects.<sup>47,73,134,141,245,266,268</sup>

There is little information regarding fertility (page 828) in patients with SCA, but reproduction rates probably are decreased. The slow, sinusoidal circulation of the placenta and the high degree of oxygen extraction provide an excellent milieu for sickling, thrombosis, and hemolysis.<sup>81</sup> The fetal risk is high, with an overall salvage rate of only about 58%, compared with 87% in normal women. The fetal wastage is due to a combination of abortions and stillbirths.<sup>134,141,245</sup> The incidence of premature birth is increased and the birth weights of viable infants are decreased.<sup>130</sup> There is no evidence of an increased incidence of congenital malformations or of particular susceptibility of the offspring to other medical disorders.<sup>245</sup>

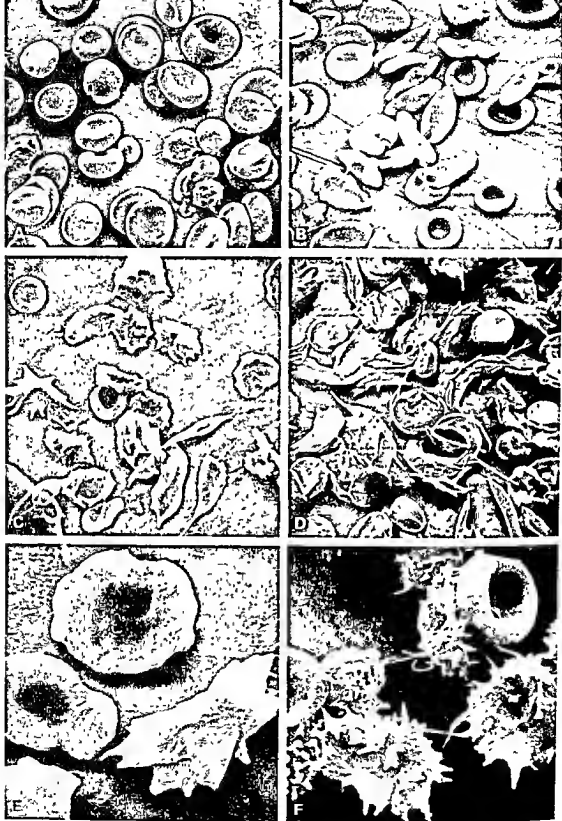
In pregnancies of patients with sickle cell trait a miscarriage rate of 9.7% was reported, as compared with a rate of 2 to 4% when one or neither parent had the sickle cell trait.<sup>277</sup>

Although the risk of serious complications during pregnancy in patients with HbSC disease and HbS- $\beta$ -thalassemia is increased, current data suggest a lower maternal mortality rate, fewer episodes of heart failure, painful crises, or puerperal sepsis, and a higher incidence of toxemia than in SCA, but the rates of puerperal infection and pulmonary disease are about equal in the two conditions. The fetal salvage rate in patients with HbSC disease and HbS-thalassemia is about 70% and birth weights are normal.<sup>135,141</sup>

### Laboratory Findings

Severe or moderately severe normocytic, normochromic anemia is present during the entire life of patients with SCA. Red cell indices usually are normal, but the MCV may be increased or reduced. It is possible to separate the red cells into several populations on the basis of cell density.<sup>29,61,293</sup> Reticulocytes are least dense, mature biconcave discs are intermediate, and the irreversibly sickled cells are most dense. The number of ISC's in the blood varies considerably from one patient to another.<sup>302</sup>

The red cells of patients with SCA show great *morphologic distortions* that are best seen when the cells are examined by phase or



**Fig 25-4.** Erythrocytes from a patient with SCA examined with scanning beam electron microscopy. **A**, Oxygenated blood. The red cells appear normal except for one microspherocyte. There are three leukocytes in the field. **B**, Oxygenated irreversibly sickled cells are smooth in texture and outline, but are ovoid or boat like in shape. **C**, Partial deoxygenation causes the cells to assume bizarre shapes with spikes, spicules, and filaments that protrude from the cells. **D**, More complete deoxygenation causes the cells to assume sickled shapes with longitudinal surface striations. **E**, At higher magnification the cells have a sculptured texture. Multiple spike-like protrusions are seen at the polar ends of one cell. **F**, Complete deoxygenation of acanthocytic sickle cells produces numerous short protrusions and occasional long filaments.

scanning electron beam microscopy (Fig. 25-4), but also are very evident on conventional blood smears.<sup>154,236</sup> (Plate XII.) Most of the poikilocytes are cigar-shaped, crescent-like, or ovalocytic, but acanthocytes and target cells are also found. Many of the poikilocytes show indentations, but scanning electron beam microscopy of the membrane has not shown a qualitative difference from normal or other cells. Nucleated red cells and red cells with basophilic stippling, diffuse polychromatophilia, Howell-Jolly bodies, Pappenheimer's bodies, and Cabot rings usually are present. The presence of large numbers of cells with inclusions suggests the combination of an accelerated rate of release of cells and a hypofunctional spleen. Examination of red cells by electron microscopy in the oxygenated unsickled state reveals the occasional presence of small, dense aggregates of hemoglobin adjacent to the membrane; these have the morphologic characteristics of small Heinz bodies.<sup>188,286</sup> The morphologic alterations are accompanied by other abnormalities, including decreased mechanical fragility and abnormalities of osmotic fragility, which reflect the variety of stages of red cell maturation and the differently shaped cells that were described above. The sedimentation rate in sickle cell anemia is consistently decreased.

Erythrokinetic studies performed in the steady state reflect a four- to five-fold increase in rates of red cell production and erythron iron turnover, and a five- to ten-fold decrease in red cell life span. As measured by <sup>3</sup>H- or <sup>32</sup>P-labeled diisopropylfluorophosphate (DFP), destruction of red cells is random rather than senescent (Chapter 5).<sup>24,26,205,215</sup> This pattern of cell loss is compatible with the observations that the number of irreversibly sickled cells (ISC) is inversely correlated with cell life span and that such cells are of various chronologic ages.<sup>302</sup> Endogenous carbon monoxide production and excretion of urobilin and urobilinogen in the urine and feces are increased.

The erythrocyte production rate may be impaired by folate deficiency,<sup>161,194</sup> infection, inflammation, the ingestion of alcohol<sup>138</sup> or medications, aplastic crises,<sup>185</sup> or the pro-



Fig. 25-8. Sickled red corpuscles from a patient with SCA. The cells were washed several times with normal saline solution and the cell suspension was then allowed to remain under oil. After time had been allowed for sickling to occur, formalin was added in order to fix the cells in their abnormal shape. A smear was then made and prepared with Wright's stain ( $\times 1050$ ) (Prepared by Dr. Irving J. Sherman.)

longed administration of oxygen at high concentration.<sup>262</sup> In the patient with a red cell life span of approximately 10 to 15 days, a decrease in rate of cell formation produces a dramatic aggravation of the anemia in a short time. Thus, a rapid fall in hematocrit may occur during an acute infection and is not necessarily caused by increased hyperhemolysis<sup>413</sup> (Chapter 24).

The red cell destruction is partially intravascular, thereby producing an elevation of plasma heme proteins and decreased haptoglobin concentration.<sup>72</sup> Serum levels of non-conjugated bilirubin are moderately increased.

The white blood cell count is often elevated, usually because of increased neutrophils. Each patient tends to maintain a typical leukocyte pattern, except during periods of painful crisis, infection, folate deficiency, or other intercurrent disease. It is important that the usual leukocyte pattern of the patient be known since leukocytosis of 16.0 to

$20.0 \times 10^9$  cells/l often is present during relatively asymptomatic periods in some patients and this may reflect "asplenia" rather than inflammatory or infectious disease.

Studies of neutrophil kinetics with DF<sup>32</sup>P indicate that the circulating granulocyte pool is normal or enlarged, but the total granulocyte pool is not increased to the same degree. This discrepancy indicates a shift from the marginal to the circulating blood granulocyte compartment (Chapter 6). The disappearance time of neutrophils is slightly reduced; the granulocyte turnover rate is increased.<sup>35</sup>

Platelet counts may be within normal limits or increased, but the number of platelets may become decreased when there is folate deficiency or during an aplastic crisis. An increase in platelet numbers suggests reduced splenic sequestration.<sup>248</sup> Megakaryocytes or megakaryocytic fragments may be found in the blood during episodes of bone marrow infarction.<sup>55</sup> The rate of platelet turnover has not been studied extensively, but the available information suggests that platelet consumption increases in patients with thrombotic disease, including those with sickle cell disorders.<sup>130a</sup> Platelet function seems to be normal. Factor VIII activity may be increased, but factors IX, XI, and XII are normal.<sup>364</sup>

The bone marrow erythroid mass is increased. Maturation of the erythroblasts is normal and there is little to distinguish the bone marrow from that found in association with other severe chronic hemolytic anemias other than the presence of sickle cells or of long filamentous strands of what appears to be erythrocyte cytoplasm. These strands may extend across the whole oil-immersion field and may be no more than 2  $\mu$ m in diameter.

Despite the prolonged period of maximal stimulation with resulting bone marrow hyperplasia, there is no evidence of bone marrow failure, nor is there evidence to indicate that the constant hematopoietic stimulation induces neoplasia. The incidence of aplastic anemia, myelofibrosis, or leukemia does not seem to be greater in SCA patients than in normal populations. Myeloma has been noted in patients with SCA.<sup>327a</sup>

## Laboratory Tests for Sickling

In SCA the number and character of sickle cells in stained blood smears may not be sufficiently striking to warrant a diagnosis. Furthermore, sickled cells are rarely, if ever, seen in blood smears from persons with sickle cell trait. However, the sickling phenomenon may be induced by a variety of maneuvers, which depend on the deoxygenation of hemoglobin. It may be induced by sealing a drop of blood under a coverslip to exclude oxygen, or by adding agents that induce chemical deoxygenation, such as 2% sodium bisulfite ( $\text{Na}_2\text{S}_2\text{O}_3$ ), or a preparation of sodium dithionite ( $\text{Na}_2\text{S}_2\text{O}_4$ ); or by gaseous displacement of oxygen by mixtures of nitrogen and carbon dioxide. The degree and rapidity of the sickling phenomenon depend primarily on the amount of sickle hemoglobin and the degree of deoxygenation.<sup>123</sup> Cells containing other hemoglobins in which the beta  $6^{\text{Olu}} \rightarrow \text{Val}$  substitution is present, such as HbC<sub>Georgetown</sub> and HbS<sub>Memphis</sub>, also exhibit the sickling phenomenon. Under conditions of severe deoxygenation, sickling takes place in cells that contain HbI; under conditions of hyperoxygenation, deer cells will undergo sickling.<sup>151</sup>

There are a number of commercially available preparations for the detection of sickle hemoglobin; these depend upon the decreased solubility of deoxygenated HbS in high phosphate buffer solutions. They have obvious advantages in laboratories in which only sporadic testing for SCA is required, but the reliability of the commercially available kits varies from excellent to poor, and, because of the cost of the commercial products, laboratories in which frequent testing for SCA is performed prepare their own reagents.<sup>283a</sup>

The available screening tests utilize the classic sickle-cell preparation described above, the solubility test,<sup>146a</sup> or hemoglobin electrophoresis (Chapter 24). Each of these tests has particular advantages and pitfalls, but the most reliable primary screening method is probably electrophoresis on cellulose acetate.<sup>217, 256</sup> This method allows detection of abnormal hemoglobins other than HbS as well as identification of some of the

doubly heterozygous forms of sickle cell disorders. Whole blood, dried blood specimens, or hemoglobin solutions may be used.<sup>97</sup> When electrophoresis is employed as a primary screening method, a solubility test may be used to identify the occasional persons who will be found heterozygous for HbD (page 846). Identification of abnormal hemoglobins in more precise fashion than that provided for by cellulose acetate electrophoresis and tests of solubility requires family studies and laboratory technology not available in the usual screening program.

### Sickle Cell Trait and Screening

The term "sickle cell trait" refers to the inheritance of one determinant for the abnormal beta 6<sup>Val</sup> HbS chain, and one determinant for normal beta 6<sup>Glu</sup> HbA. The cells of such individuals contain both HbA and HbS but have more HbA than HbS.<sup>37</sup> The ratio of HbA to HbS is related to the synthetic rates of hemoglobins A and S rather than to selective destruction of one of them.<sup>28</sup>

Red cells containing both HbS and HbA require much greater deoxygenation than do those from patients with sickle cell anemia (HbSS) before sickling occurs. As mentioned above, sickled cells are rarely found in the blood of a person with the sickle cell trait<sup>121</sup> and there are few established complications attributable to this trait.<sup>71</sup> Hyposthenuria,<sup>23</sup> spontaneous hematuria,<sup>46,112</sup> renal papillary necrosis,<sup>127</sup> priapism, central retinal artery occlusion,<sup>69</sup> splenic infarction,<sup>231,310</sup> splenic rupture with bacterial endocarditis, and greater frequency of asymptomatic bacteriuria in women than in the general population<sup>346</sup> have been reported in persons with the sickle cell trait. Retrospective studies show the same overall mortality rate in persons with the sickle cell trait as in normal persons.<sup>12,132</sup> However, complications may be more frequent and illness may be more severe among patients with the sickle cell trait and pulmonary disease, thrombophlebitis, or diabetic vascular disease than in those without this trait.<sup>132</sup>

Four deaths among soldiers undergoing strenuous physical conditioning at an altitude

of 4060 feet were attributed to the sickle cell trait.<sup>160</sup> In areas where cerebral malaria (*Plasmodium falciparum*) is endemic, the mortality rate among infants and young children with the sickle cell trait is less than in those with HbA<sup>7</sup> (page 813). In temperate climates, however, the sickle trait seems to confer no physiologic advantages.

A number of large-scale screening programs designed to detect heterozygotes for abnormal hemoglobins and to inform them of their hemoglobin type have been carried out, primarily among black populations.<sup>182,274,275,344</sup> The major purpose of such programs is to provide affected individuals the opportunity to determine the probability of having a child with homozygous disease. Effective education of those who are screened is necessary in order to make clear to them the mode of inheritance of abnormal hemoglobin genes and the difference between heterozygosity and homozygosity. Screening programs not associated with effective counseling will sow confusion and may even be harmful, especially for the well child who is presented with the "stigma" of having an inherited illness. Furthermore, since the interaction of other abnormal hemoglobins or thalassemia with HbS may have serious consequences, any screening program designed to provide genetic counseling should employ techniques that will detect these conditions as well.

### Morbid Anatomy

In SCA the bone marrow is hyperplastic, and extramedullary hematopoiesis frequently is evident in the lungs, liver, and retroperitoneal and perirenal regions. Hemosiderosis of varying severity is present in the liver, the spleen, kidneys, lymph nodes, bone marrow, and heart, even in patients who have received relatively few transfusions. Various phases of tissue infarction and reparative fibrosis may be observed in almost any tissue.<sup>81,89,169,316</sup>

The heart is almost always dilated and enlarged. Hypertrophy of fibers and a variety of nonspecific degenerative changes are found in the myocardium, together with extensive *myofibrillar* fibrosis involving the right and left

ventricular muscle and the interventricular septum. The nuclei of muscle cells appear normal, but there are paranuclear deposits of hemofuscin pigment.<sup>17a,191</sup> Cardiac hemosiderosis, so common in thalassemia, is less striking in patients with SCA. The endocardium is typically free of lesions, as are the coronary arteries and large veins of the heart. When pericarditis is present it is usually due to other disease processes or is secondary to pulmonary or pleural infection.

The *lungs* show acute and chronic changes that are the result of infection, infarction, and cardiopulmonary failure.<sup>42,81</sup>

In the *kidneys* there are dilated glomerular and peritubular capillaries that are engorged with sickled cells, as well as enlarged glomeruli, foci of interstitial hemorrhage with hemosiderosis, scars of infarcts, and papillary necrosis.<sup>10,46,81,252,337</sup> In addition, abnormalities of the glomerular basement membrane with or without glomerular fibrosis as well as anatomic changes similar to those of proliferative glomerular nephritis have been described.

The *liver* usually is enlarged and the lesions include engorgement of the sinusoids with sickled erythrocytes, hemosiderosis, and enlarged Kupfer cells with red cell phagocytosis.<sup>271</sup> Massive infarction of the liver has been occasionally encountered and there are varying degrees of cellular infiltration and fibrosis.

The *spleen* is enlarged in childhood and is fibrotic in adulthood. The splenic sinusoids are dilated and the littoral reticuloendothelial cells are hyperplastic. Erythrophagocytosis is prominent and irreversibly sickled cells are present in the sinusoidal spaces. By light and electron microscopy these appear as dense, dark cells. Findings in the spleens of children are similar to those noted in other forms of hemolytic anemia in which red cell entrapment is prominent.<sup>78,327</sup>

### Management and Therapy

Because the natural course of sickle cell anemia has never been comprehensively documented, the long-term results of many therapeutic manipulations cannot be adequately

evaluated. Many medications and manipulations have been touted as beneficial, only to be found ineffective or even deleterious to health upon prolonged or more careful study. Programs directed at the education of groups of laymen as well as patients, together with adequate screening and genetic counseling, may be expected to have an impact on the management of the sickle cell disorders.<sup>119,314</sup> The identification of adult persons with sickle cell trait will permit informed decisions to be made about family planning. Recognition of sickle cell disease at the time of birth may allow the prevention of some of the complications and catastrophes that occur in early childhood.<sup>53</sup> The psychosocial aspects of management are complicated and multifactorial; although of great importance, they are not susceptible to simple guidelines.<sup>350a</sup> Many patients are physically active and lead nearly "normal" lives during their earlier years, except when specific and serious complicating illnesses, such as pneumonia or splenic sequestration, occur or painful sickle cell crises develop. The diagnosis of sickle cell crisis requires exclusion of the possibility of other disease, as discussed earlier (page 829), and identification of precipitating causes. Many medications have been used, but no single nonanalgesic, noncontroversial agent has been described.

### Blood Transfusion

The single, least controversial, and most effective therapeutic measure available is the transfusion of normal red cells. Repeated transfusions, or repeated partial exchange transfusion therapy, has been advocated and has been effective in the prevention of painful episodes and in the enhancement of growth.<sup>32,33,315</sup> Transfusion probably is of benefit in the management of splenic sequestration, priapism, renal epistaxis, surgical procedures, and cardiovascular and other life-threatening complications. However, repeated transfusion therapy for prophylactic purposes is not a simple matter because of problems in the acquisition of compatible blood, transfusion reactions, and the hazards of hepatitis and iron overload. For these rea-

sons, transfusions are best reserved for the more severe episodes of clinical illness or for patients who have severe disease and almost constantly recurrent symptoms. Exchange transfusions may provide the advantages over single transfusion in that smaller increases of blood volume, minimal additions to iron overload, and perhaps greater numerical gain in the ratio of normal cells to sickle cells are produced.

### Other Approaches to the Treatment of Sickle Cell Crises

Since painful episodes are precipitated by infection, fever, dehydration, acidosis, and perhaps by cold as well,<sup>273a</sup> measures that prevent or remedy these conditions have been used. Hydration is essential; induction of alkalosis has been stated to be of benefit by some<sup>15,116</sup> but others have refuted this claim.<sup>269</sup> Carbonic acid anhydrase inhibitors have been used with the expectation of decreasing intracellular carbonic acid, but such treatment has not been of clinical benefit.<sup>99</sup>

The pharmacologic approach to the treatment of painful crises has included attempts to modify HbS chemically in order to minimize hemoglobin molecular polymerization and its consequences. Conversion to methemoglobin of a part of the HbS by oral administration of nitrates has not been particularly helpful.<sup>32</sup> Attempts to affect sol-gel transformation at the hemoglobin-membrane interface and to alter the cell membrane have included the use of phenothiazines,<sup>130,207,235</sup> progesterones,<sup>146</sup> testosterone,<sup>210</sup> and other steroidal hormones.<sup>2</sup> Clinical trials<sup>130,146,210,233</sup> give conflicting impressions regarding their efficacy. Magnesium sulfate,<sup>15</sup> hyperbaric oxygen,<sup>76,182,263</sup> and dextrans,<sup>14,103,234,340</sup> have been used, but are not clearly beneficial. Attempts to minimize cell aggregation and vascular thrombosis with dextrans,<sup>14,103,234,340</sup> with anticoagulants,<sup>170,264,280</sup> and with agents that deplete coagulation proteins (Arvin)<sup>106</sup> have been made, but none of these has proven value and their use over prolonged periods carries significant risks. HbS cells treated with nitrogen mustard have decreased propensity to sickle and have

normal oxygen association characteristics.<sup>272</sup> However, the concentration of nitrogen mustard needed to inhibit sickling precludes its *in vivo* use.<sup>53</sup>

Orally administered urea has been advocated for the prevention of painful episodes and intravenously administered urea, for the alleviation of pain and the prolongation of cell survival.<sup>204,205</sup> The rationale for urea therapy was based on the hypothesis that hemoglobin polymerization depends on intramolecular hydrophobic bonding and the fact that urea can disperse such bonds.<sup>227,228</sup> Interference of gelation of deoxy-HbS solutions by urea has been demonstrated,<sup>9</sup> and inhibition of sickling as well as the effects of urea on the oxyhemoglobin dissociation curve have been described.<sup>43</sup> Studies of the *in vitro* effects of urea have shown a decrease in viscosity of deoxy-HbS solutions and inhibition of the sickling phenomenon by 0.4 M urea (equivalent to BUN of 1120 mg/dl), but not by 0.1 M urea (equivalent to BUN of 280 mg/dl).<sup>296</sup> These studies have not substantiated claims of *in vitro* inhibition of sickling by much lower concentrations of urea such as 0.01 M (equivalent to BUN of 28 mg/dl).<sup>196,227,228</sup>

Uncontrolled preliminary clinical studies showed conflicting results, with benefit in some<sup>206,227,228</sup> but not in others.<sup>192,195</sup> A clinical cooperative controlled study of the effect of the intravenous administration of urea in invert sugar demonstrated no benefit from urea as compared with alkali or with simple hydration.<sup>177</sup> Because of these studies, most investigators believe that urea is not a therapeutically valuable agent. Moreover, administration of urea may be associated with accelerated hemolysis,<sup>25,192</sup> and may produce a potentially dangerous diuresis.

Potassium cyanate inhibits sickling by different mechanisms than that proposed for urea.<sup>50,51,74,77,178,198,201</sup> One mechanism is the specific inhibition of polymerization as the result of carbamylation of the N-terminal group of the hemoglobin polypeptide. Perhaps a more important effect is an increase in the oxygen affinity of the carbamylated hemoglobin. Because of the increased oxygen affinity, there is less deoxy-HbS at a given

oxygen tension and less propensity for sickling.<sup>215</sup>

HbS cells have been treated *in vitro* with cyanates<sup>107,152,180,216</sup> and have shown prolonged survival upon reinfusion. Patients treated with oral sodium cyanate in amounts of 20 to 30 mg/kg/day have been found to have increased red cell survival times, but clinical improvement has not been impressive.<sup>53,250</sup> There are insufficient data regarding cyanate therapy to provide estimates of its value or to make suggestions concerning indications for its use. The long- and short-term toxic effects of cyanate at various dose levels, including hepatotoxicity in rats<sup>108,230</sup> and dose-related motor peripheral neuropathy observed in patients and in dogs, require careful evaluation.

Administration of oxygen to patients in crisis offers little if any benefit, but oxygen should be given to patients with ancillary cardiopulmonary disease.

### Treatment of Complications

The complications of sickle cell disease are numerous, and many require surgical care. When general anesthesia is to be used, partial exchange transfusion may be preferable to standard blood transfusions, in order to avoid blood volume overload. There is no evidence

that high hemoglobin concentrations improve the healing process.<sup>53</sup> Furthermore, the dangers of cardiac failure or pulmonary disease during the surgical-anesthetic event are enhanced by an overexpanded blood volume. Transfusions usually are not needed when local or spinal anesthesia is used. When orthopedic operations are performed and tourniquets must be used to maintain hemostasis, exchange transfusions are necessary.

*Ankle ulcers* (Fig. 25-6) are distressing and quite common. They have been classified in various ways and possibly require different types of management.<sup>332</sup> Bed rest, elevation of the affected extremities, and wet-to-dry dressings are initial forms of therapy. Antibiotics should be administered systemically if septicemia or extensive local infections are present. Maintaining a normal Hb level for some months by transfusion and grafting with a fairly thick split-thickness graft may be beneficial.<sup>332</sup> Zinc deficiency has been suggested as contributing to ulceration<sup>301a</sup> and there are reports of beneficial effects of oral therapy with zinc sulfate in patients with venous stasis ulcers and low serum Zn levels.<sup>122a</sup> Occlusive gel boots (Unna boots) are used when the acute inflammation has subsided, and the patient then may assume partial ambulation. Because of the tendency to slow healing and the recurrent nature of the

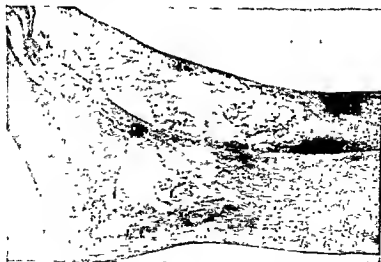


Fig 25-6. Chronic leg ulcers in a patient with SCA (Courtesy of Dr. A. F. Jonas, Jr.)



leg ulcers, gradual ambulation and the prophylactic use of lightweight support stockings are advisable.

The multiple osseous disorders that occur may require orthopedic surgical procedures. Aseptic necrosis of the hip may be managed without surgical therapy, but in patients with severe disease either arthroplasty or total hip replacement may be necessary.<sup>63,90</sup>

The ocular involvement in SCD may include neovascularization of the retina with retinal tears, and such lesions may require photocoagulation or surgical intervention. Exchange transfusion is usually indicated prior to ocular surgery.<sup>11</sup>

Whether or not all patients with SCD should receive daily supplemental folic acid is uncertain, but evidence of folate deficiency does occur, either with or without demonstrable clinical effects or megaloblastic anemia.<sup>161,194,241,256,341</sup> It seems judicious to give 0.3 mg of folic acid daily, especially to patients who are pregnant or to those who have decreased folic acid sources.

## Hemoglobin C Disorders

### Hemoglobin C

In HbC, lysine replaces glutamic acid in position 6 of the  $\beta$ -chain (Chapter 4, page 174). This abnormal hemoglobin is more stable in reduced form than is normal hemoglobin and is more soluble than HbA. However, under conditions that permit partial drying and partial hemolysis, HbC may crystallize in vitro, provided its concentration is sufficiently great (more than 44%).<sup>57</sup> Thus, tetragonal crystals of hemoglobin were demonstrable when suspensions of washed erythrocytes in 3% sodium citrate solution were sealed under a coverslip, left at room temperature for hours, and allowed to dry slowly. Intraerythrocytic crystals of HbC have been observed in blood smears of individuals with HbC disorders and such red corpuscles may assume a rigid rod-shaped form.<sup>82,84,187</sup> This phenomenon, however, is not unique for HbC and has been reported to occur with a wide variety of other hemoglobinopathies.<sup>187</sup>

The HbC gene is found in 2 to 3% of the

Negro population in the United States. About one in 6000 persons have HbC disease (Table 24-7, page 813). In Africa, the gene distribution is very unusual. There is a 14% gene frequency in Northern Ghana and the Upper Volta, with a rapidly declining incidence in all directions from there.<sup>193,275</sup> However, sporadic instances of HbC trait and even of HbC disease<sup>102</sup> have been reported in individuals in whom African ancestry was improbable.

HbC trait (HbAC) is characteristically an asymptomatic state, with the possible exception of the rare occurrence of hematuria or priapism.<sup>269</sup> Target cells are found in association with HbC trait in various numbers, but anemia is not present and there is no evidence of increased blood destruction. No evidence of gene interaction was observed when HbC trait was found in association with pernicious anemia, glucose 6-phosphate dehydrogenase deficiency, or hereditary elliptocytosis.<sup>312</sup> The HbC trait does not seem to complicate other diseases or make them more severe, except for  $\beta$ -thalassemia minor (page 873) and HbS trait (page 844).

### Homozygous HbC Disease (HbCC)

The usual clinical picture of HbC disease includes mild intermittent abdominal discomfort, occasional arthralgia without overt arthritis, intermittent mild jaundice, splenomegaly, and, occasionally, hematuria in a Negro who otherwise is quite well.<sup>128,163,164,258,312,329,347</sup> The disease usually is discovered when medical attention is sought for unrelated symptoms or during the conduct of a hemoglobin screening program.

The few symptoms of HbC disease are not aggravated by other diseases, except possibly those that cause dehydration and hyperosmolality of the blood, such as diabetic hyperglycemia. These conditions may cause intraerythrocytic dehydration with formation of intraerythrocytic hemoglobin crystals and an increase in the usually minimal rheologic abnormalities of blood flow. Cholelithiasis is said to be more common in individuals with this disease than in normal persons. Unlike sickle cell disease, there is no known in-

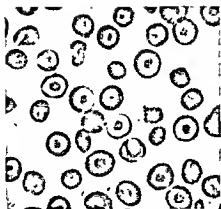


Fig. 25-7 Photomicrograph of stained smear from a patient with homozygous C disease (HbCC) showing the characteristic target cells

creased morbidity or mortality associated with pregnancy.

The red cells are strikingly abnormal (Fig 25-7). A mild hemolytic anemia with target cells, microspherocytes, and occasional crystal-containing cells<sup>3,57,84</sup> is associated with decreased erythrocyte cation content, abnormality of cell water,<sup>225</sup> and a tendency to increased intracellular viscosity with decreased cell deformability. The formation of hemoglobin aggregates and of crystals is thought to account for the rigidity of the red cells, their decreased filtrability, and possibly their fragmentation with formation of the dense microspherocytes that are always present.<sup>155,157</sup> In the absence of the spleen, these changes are especially striking. Erythrocyte osmotic fragility curves are abnormal, indicating the presence of populations of excessively fragile cells (microspherocytes) as well as resistant ones (target cells).<sup>159</sup>

Together with a modest shortening of red cell life span, there is constant mild reticulocytosis and normoblastic hyperplasia of the bone marrow. Erythrokinetic studies show increased rates of plasma iron turnover and incorporation of iron into the red cell mass<sup>156,325</sup>—findings consistent with the degree of hemolysis. There is no evidence of increased destruction of cells in the bone marrow and, although the spleen sequesters cells, there is little clinical benefit from splenectomy. There is no distinctive morbid anatomic feature that distinguishes HbC disease

from other varieties of modest chronic hemolytic anemia.<sup>156</sup> Therapy is neither available nor needed. The entity should be recognized in order to avoid unnecessary diagnostic and therapeutic procedures.

### Sickle Cell-Hemoglobin C Disease

Sickle cell-hemoglobin C disease results from the inheritance of a HbS gene from one parent and of a HbC gene from the other parent (Chapter 24). This doubly heterozygous condition is found predominantly in Ghana and in the New World. Among Negroes, the disease has an approximate frequency of 1:833 births in the United States<sup>221</sup> (Table 24-7) and 1:1400 births in Jamaica.<sup>300</sup> In Ghana, the disorder is about as common as sickle cell anemia, and in some regions affects 25% of the population.<sup>174</sup>

The disorder is similar in clinical presentation to SCA, but is of lesser severity and is more compatible with longevity. Several series of patients have been reported—from the United States,<sup>263</sup> Africa,<sup>175</sup> and Jamaica<sup>271</sup>; in these the clinical features were similar but in all there was a wide range of clinical severity.

Growth, body habitus, and sexual development are nearly normal. The cardiovascular changes that are so common in SCA are minimal. In about 40% of the patients in one series<sup>300</sup> the disease was only discovered after its subjects were 20 years of age. The most common complaint is musculoskeletal pain, which is mild to moderate, may be widespread and symmetrical, and may be limited to one portion of the affected bone or bones. The relatively benign course of HbSC disease is attended by several complications that are said to be more common than in SCA. Aseptic necrosis of the femoral heads (Fig. 25-8) has been observed in a high proportion of the patients.<sup>239a</sup> Acute pulmonary disease was found to be common in Jamaica<sup>300</sup> and in the United States.<sup>268</sup> Splenomegaly was present in about two thirds of the adult patients in both series. Symptomatic splenic infarction and the splenic sequestration syndrome occur even in adults. It has been stated that complications of pregnancy are more common

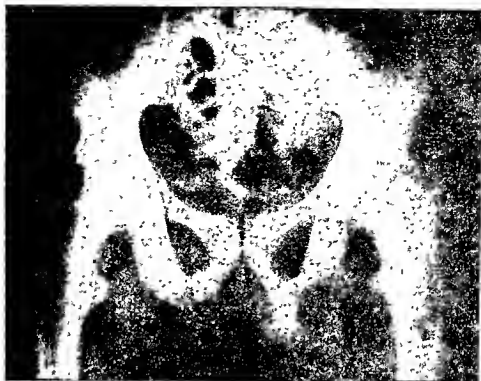


Fig. 25-8. Aseptic necrosis of the femoral heads in a patient with sickle cell hemoglobin C disease. There is irregular erosion of the articular cartilage, erosion of the superior cortical margin of the femoral heads, and a mixture of radiolucency and increased density throughout the femoral heads.

and severe in HbSC disease than in SCA, but the evidence is conflicting (see page 835).

A high incidence of ocular involvement was not emphasized in earlier series, but retinal vascular disease was present in almost all of the Jamaican patients and one third had retinitis proliferans. An analysis of age-matched groups of patients with SCA and sickle cell-hemoglobin C disease revealed that retinal disease was more severe in the latter group.<sup>67</sup>

About 20% of patients with sickle cell-hemoglobin C disease have leg ulcers at one time or another. Hepatomegaly is present in about 40% and clinically detectable jaundice is uncommon, but mild hyperbilirubinemia is frequent. There is usually a modest anemia; only about 10% of the patients have Hb levels less than 10 g/dl. Transient, more severe anemia can develop in the presence of bleeding or infection. The cells are normocytic, normochromic and blood films contain many target cells, but few irreversibly sickled cells. On blood smears and in wet preparations

there are occasional cells that contain condensed hemoglobin crystals that are dark and have parallel sides with a pyramidal or rounded shape<sup>84</sup> (Fig. 25-9). These crystals presumably are formed from both S and C hemoglobin molecules. Although it is not possible to distinguish clearly between the sickled cells of patients with SCA and those with sickle cell-hemoglobin C disease, the latter often form quite characteristic double aggregates within the cell. Reticulocyte counts are only modestly increased, there is normoblastic hyperplasia of the bone marrow, with an increased rate of red cell production and decreased red cell life span.<sup>222</sup>

Although the course of SC disease is benign as compared with that of SCA, occasionally neurologic lesions and episodes of priapism or hematuria occur, in addition to the clinical manifestations previously described. Most of the patients have reasonably good health, however, and the disorder is compatible with long life. The disease may remain undiagnosed, or may be detected dur-

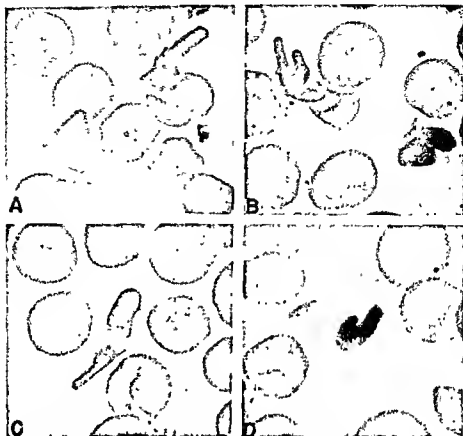


Fig 25-9 Photomicrographs of stained blood smears of patients with HbSC disease. Dark, blunt protuberances and other distortions produced by condensation of hemoglobin crystals are seen. In C the red corpuscle is elongated and the hemoglobin is concentrated at each end, leaving a hemoglobin-free central area. (From Diggs and Bell<sup>22</sup> courtesy of the authors and Grune & Stratton, Inc.)

ing the course of a family study, or during pregnancy.

## Hemoglobin D

Hemoglobin D has the same electrophoretic mobility at alkaline pH on conventional media as does sickle hemoglobin.<sup>199</sup> HbD is distinguished from HbS by agar gel electrophoresis, at pH 6.2, by its normal solubility, and by the fact that it does not cause sickling. Although there are a number of hemoglobins D with  $\beta$ -chain mutations (eg, D Bushman [16 Gly  $\rightarrow$  Arg], D Ibadan [87 Thr  $\rightarrow$  Lys], and D Punjab [121 Glu  $\rightarrow$  Gln], also known as D Los Angeles, Cyprus, Conley, Chicago, Portugal), only D Punjab occurs commonly.<sup>199,276</sup> Alpha-chain abnormalities that

have the electrophoretic mobility of HbD include HbD St Louis and HbD Washington, which are the same as HbG Philadelphia ( $\alpha_2^{65} \text{Asn} \rightarrow \text{Lys}$ ). These hemoglobins cannot be separated, one from another, by electrophoresis. HbD Punjab probably arose in India, and is found among American Negroes about once in 2500 individuals.<sup>276</sup> Persons with HbD are usually found by means of screening examinations and, without electrophoresis at acid pH, sickling tests, or solubility tests, they would be mistaken for persons with the sickle cell trait. Except for hematuria, no clinical, hematologic, or physiologic abnormalities are associated with the hemoglobin D trait (HbAD).<sup>119</sup> Homozygous HbD disease is rare. It may be associated with mild hemolytic anemia, but there are virtually no

symptoms. There is no splenomegaly, but target cells and spherocytes are found.<sup>324</sup>

### Sickle Cell-Hemoglobin D Disease

Sickle cell hemoglobin D disease is uncommon.<sup>148,311</sup> It is clinically indistinguishable from the more mild cases of SCA. HbS and HbD interact and produce hemoglobin polymerization, the sickling phenomenon, and the previously described findings of SCA.<sup>49,267,285</sup> Since these patients have sickled erythrocytes and hemoglobin that migrates in the HbS position at pH 8.6, they may be misdiagnosed as having SCA. The clinical characteristics are not uniform, but all the subjects have hemolytic anemia. Some patients have had severe disease and others were almost asymptomatic.<sup>321</sup>

HbD is easily separated from HbS by agar gel electrophoresis at pH 6.2. HbD-thalassemia with clinical disease resembling SD disease was reported in a Persian girl<sup>143</sup> as well as in a family with  $\beta$  thalassemia-HbD.<sup>163</sup>

## Hemoglobin E

Hemoglobin E has an electrophoretic mobility slightly anodal to HbC at pH 8.6 and is about equal in solubility to HbA. The abnormality results from the substitution of lysine for glutamic acid at the 26th amino acid of the  $\beta$ -polypeptide chain (Table 24-1). HbE is found in a high proportion of Thais, (13.6%),<sup>60</sup> in Burmese (15.3%),<sup>64</sup> in Cambodians (35%),<sup>45</sup> and in some Indonesian, Ceylonese, and Malaysian (1%) populations. Significantly higher frequencies have been observed in highly malarious areas of Thailand than in neighboring regions where there is a low incidence of malaria.<sup>100</sup> Elsewhere than in Asia, occasional cases have been encountered.<sup>5,335a</sup> HbE<sub>Saskatoon</sub> ( $\beta^{226} \text{Glu} \rightarrow \text{Lys}$ ) has been reported in individuals of Scottish origin.<sup>335a</sup>

The heterozygous abnormality, HbE trait (HbAE), is asymptomatic. There is no sickling, minimal hypochromia, no target cells, and there are no inclusion bodies or alterations of osmotic fragility. About 35 to 49%

of the hemoglobin is HbE, the remainder being HbA.<sup>338</sup>

### Homozygous HbE (EE) Disease

The homozygous state (HbEE) is quite common among Asiatic populations and is characterized by a mild microcytic, normocytic anemia with 25 to 60% target cells, decreased osmotic fragility, and minimal signs of hemolytic anemia. Splenomegaly is absent or slight. Approximately 92 to 98% of the hemoglobin in the erythrocytes is HbE. The quantity of HbF is normal or slightly increased.<sup>60,190a</sup> HbC disease and HbE disease may be difficult to distinguish one from the other, but, in addition to the racial differences of the subjects, usually more target cells are present and splenomegaly is more pronounced in persons with HbC disease than in those with HbE disease. HbE can be distinguished from HbC by acid agar gel electrophoresis and by various chromatographic techniques, or by "fingerprinting" (Chapter 24).

## HbS-O Arabia

The substitution of HbO Arabia ( $\beta^{121} \text{Glu} \rightarrow \text{Lys}$ ) causes a charge difference which is similar to that of HbC. On routine electrophoresis this hemoglobin migrates to that position.

Patients with HbS and HbO Arabia have been reported from the Sudan,<sup>144,257</sup> the United States,<sup>150</sup> and Jamaica.<sup>217</sup> The clinical manifestations are such that, on routine examination and electrophoresis, they would easily be mistaken for those of hemoglobin SC disease.

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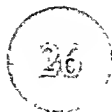


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# *The Thalassemias and Related Disorders—Quantitative Disorders of Hemoglobin Synthesis*

## Introduction

### Introduction

Genetic Mechanisms Resulting in Thalassemia

Pathologic Physiology

The  $\beta$ - and  $\delta\beta$ -Thalassemias

Homozygous  $\beta$ -Thalassemia

Thalassemia Intermédia

Heterozygous  $\beta$ -Thalassemia

( $\delta\beta$ )-Thalassemia

Miscellaneous Varieties

$\delta$ -Thalassemia

Association of  $\beta$ -Thalassemia with the Hemoglobinopathies

The Hemoglobin Lepore Syndromes

Hereditary Persistence of Fetal Hemoglobin (HPFH)

The  $\alpha$ -Thalassemias

Heterozygous  $\alpha$ -Thalassemia

Hb Bart's

Hemoglobin H Disease

Hb H or Bart's in Acquired Disorders

$\alpha$ -Thalassemia in Association with  $\alpha$ -Chain and  $\beta$ -Chain Variants

Genetics of the  $\alpha$ -Thalassemias

Treatment

Geographic and Racial Distribution of Thalassemia

Diagnosis and Differential Diagnosis of Thalassemia

The early observations regarding the beginnings of molecular biology, and their relation to the hemoglobinopathies and thalassemia, were briefly alluded to in Chapter 24. Even before homozygous and heterozygous forms of thalassemia were recognized, observers<sup>98,146</sup> noted considerable variation in the manifestations and severity of the disease; thus, terms such as "thalassemia intermedia" and "thalassemia major," to represent the clinically severe homozygous state, and "thalassemia minor" and "thalassemia minima," to refer to the considerably milder, heterozygous form, were introduced. It is now recognized that thalassemia comprises a heterogeneous group of inherited disorders of hemoglobin synthesis. Indeed, it can no longer be said that the presence of hypochromic, microcytic red corpuscles which are not the result of iron deficiency and whose osmotic fragility is decreased is the *sine qua non* of thalassemia. The morphologic picture varies in the different thalassemia syndromes, even to the point of total absence of abnormal

morphologic features or clinical manifestations in some heterozygotes.

The one common denominator of all the thalassemia syndromes is a decreased rate of synthesis of one or more hemoglobin polypeptide chains.<sup>50</sup> This primary feature is a quantitative one and contrasts with the qualitative changes in hemoglobin structure that characterize the hemoglobinopathies. Included under the category of thalassemia are all the inherited varieties of quantitative impairment of production of the normally occurring fractions of adult hemoglobin, as

well as persistence into adult life of fetal hemoglobin, and the Lepore hemoglobins, which are produced by unequal crossing over between the  $\delta$ - and  $\beta$ -genetic structural loci.

The thalassemia syndromes are classified according to the polypeptide chain or chains involved and will be described under those headings.

The clinician, confronted by the long list of possible forms of thalassemia presented in Table 26-1, may be taken aback. Fortunately, he need not be. His task is to determine whether he is dealing with one of the thalas-

Table 26-1. Classification of  $\beta$ - and ( $\delta\beta$ )-Thalassemias

Genotype	Clinical	Hemoglobin Pattern			
		Hb A <sub>2</sub> %	Hb F %	Hb A %	Other Hemoglobins
$\delta\beta$ $\delta\beta$	Normal	2.5-3.0	0	97	none
<i>Heterozygous <math>\beta</math> chain defects</i>					
(1) $\delta\beta$ $\delta^{\text{thal}}\beta$	Nil	Low	0	100	0
(2) $\delta\beta$ $\delta^{\text{fthal}}\beta$	Nil	2.5-3.0	0	>90	0
(3) $\delta\beta$ $\delta\beta^{\text{thal}}$	Thalassemia minor	3.5-7.5	1-5	>90	0
(4) $\delta\beta$ $\delta\beta^{\text{fthal}}$	Thalassemia minor	3.5-7.5	SI inc in some	94	0
(5) $\delta\beta$ ( $\delta\beta$ ) <sup>thal</sup>	Thalassemia minor	2.5-3.0	5-20	<80	0
(6) $\delta\beta$ ( $\delta\beta$ ) <sup>Lepore</sup>	Thalassemia minor	1.2-2.6	1.3-14	+	Lepore 6-15%
<i>Homozygous <math>\beta</math> chain defects</i>					
(7) $\delta\beta^{\text{thal}}$ $\delta\beta^{\text{thal}}$	Thalassemia intermedia	SI inc	6-12	>85	0
(8) $\delta\beta^{\text{thal}}$ $\delta\beta^{\text{fthal}}$	Cooley's anemia† or intermedia	Variable	20-80	+	Free alpha chains
(9) $\delta\beta^{\text{thal}}$ $\delta\beta^{\text{fthal}}$	Cooley's anemia	Variable	Almost all	0	Free alpha chains
(10) $\delta\beta^{\text{thal}}$ ( $\delta\beta$ ) <sup>thal</sup>	Thalassemia intermedia (mild)	1	60-99	0 or +	0
(11) ( $\delta\beta$ ) <sup>thal</sup> ( $\delta\beta$ ) <sup>thal</sup>	Thalassemia intermedia†	0	100	0	0
(12) ( $\delta\beta$ ) <sup>Lepore</sup> ( $\delta\beta$ ) <sup>Lepore</sup>	Cooley's anemia	0	75	0	Lepore
<i>Doubly heterozygous <math>\beta</math> chain defects</i>					
(13) $\delta\beta^{\text{thal}}$ $\delta\beta^{\text{fthal}}$	Similar to sickle cell anemia§ but usually milder	inc	10	20	70% S
(14) $\delta\beta^{\text{thal}}$ $\delta\beta^{\text{fthal}}$	Similar to sickle cell anemia§	inc	10	0	90% S
(15) $\delta\beta^{\text{thal}}$ ( $\delta\beta$ ) <sup>thal</sup>	Similar to sickle cell anemia§ but usually milder	+	+	0	S

$\beta$  refers to the normal gene for  $\beta$  chain synthesis,  $\delta$  to the normal gene for  $\delta$ -chain synthesis,  $\beta^{\text{thal}}$  to the  $\beta$  thalassemia gene with some  $\beta$ -chain production,  $\beta^{\text{fthal}}$  to the  $\beta$  thalassemia gene with no  $\beta$ -chain production ( $\delta\beta$ ) indicates that both  $\delta$ - and  $\beta$ -chain synthesis are affected

\* Silent  $\beta$  thalassemia gene

† "True"  $\beta$ -thalassemia  $A_2\beta$  thalassemia thalassemia type 1

‡ ( $\delta\beta$ ) Thalassemia, F thalassemia thalassemia type 2

§ Sickle cell thalassemia

|| In the hemoglobin pattern columns + means present

semia syndromes, rather than iron-deficiency anemia, one of the hemoglobinopathies, or another condition from which thalassemia must be distinguished, as discussed on page 883. In general, heterozygous inheritance of a thalassemia gene produces thalassemia minor or minima, or no clinical manifestations at all, while homozygous or doubly heterozygous thalassemia produces Cooley's anemia or thalassemia intermedia. From a practical, clinical standpoint it is not necessary for the clinician to identify the genotype of his patient in accordance with the lists given in Tables 26-1 and 26-3. He can and should carry out family studies in order to identify other members of the family who have anemia, splenomegaly, or other features attributable to thalassemia. This will avoid confusion at later times. Family studies, however, can be carried out by relatively simple means. More specific identification of the genotype is a task for the specialized laboratory.

### Genetic Mechanisms Resulting in Thalassemia

The present widely accepted concept of the genetic mechanisms that result in thalassemia is derived in part from genetic studies, especially of individuals doubly heterozygous for thalassemia and one of the abnormal hemoglobins, such as Hb S, and also from the application of simple and reliable methods for the separation and isolation of the globin chains of hemoglobin. The latter methods have made possible measurement of the in vitro rate of globin chain production in reticulocytes of patients with different forms of thalassemia.

It was observed, for example, that in individuals heterozygous for the  $\beta$ -thalassemia gene and for Hb S, the Hb S level was 58 to 94%, in contrast to the 25 to 47% usually found in carriers of the Hb S trait.<sup>53</sup> This was interpreted as indicating that the  $\beta^{\text{thal}}$  mutation selectively suppressed the synthesis of  $\beta$ -chains directed by that locus, whereas the other, (trans)  $\beta^S$ , gene had not been inhibited. The term "interaction" is used to describe the reversal of the normal hemoglobin A/S ratio which occurs when the  $\beta$ -thalassemia gene is

found in association with the sickle cell gene.

Unbalanced synthesis of the globin chains has been demonstrated in peripheral blood reticulocytes by measurement of the incorporation of radioactive amino acids into the globin chains.<sup>2</sup> In homozygous  $\beta$ -thalassemia, the  $\beta/\alpha$ -incorporation ratio was found to be less than 0.2; in the heterozygous state, 0.5; and in normal subjects, 1.0<sup>33,34</sup> (Fig. 26-1). There have been conflicting reports regarding  $\beta/\alpha$  synthetic ratios in the erythroid precursors of the red corpuscles in bone marrow in  $\beta$ -thalassemia, ranging from equal synthetic ratios in the heterozygote cells<sup>30,46</sup> to marked imbalance in the homozygote.<sup>48</sup> The reported discrepancies have been explained in various ways<sup>9,38</sup> and obviously deserve further investigation. Imbalance between  $\alpha$ - and  $\beta$ -chain synthesis also has been described in Hb H disease and between  $\alpha/\gamma$ -chain synthesis in homozygous ( $\delta\beta$ )-thalassemia.<sup>48</sup>

In the majority of patients with thalassemia the quantity of  $\beta$ -polypeptide chain ( $\beta^{\text{thal}}$ ) or  $\alpha$ -chain ( $\alpha^{\text{thal}}$ ) is reduced, but the amino acid sequence of the chain is normal. In some subjects there is even complete absence of gene product; that is, one of the polypeptide chains is totally lacking.<sup>57</sup> However, in the Lepore hemoglobins the polypeptide chain is abnormal, but this is merely in the sense that it represents a fusion of otherwise normal parts of the  $\delta$ - and  $\beta$ -polypeptides to form a chain of normal length. Structural abnormalities are also found in Hb Constant Spring (page 881).

An early finding was the fact that, in the total or partial absence of the  $\beta$ -peptide chain, the amount of  $\delta$ -chain or  $\gamma$ -chain is increased with increased levels of Hb A<sub>2</sub> or Hb F. This observation has proved helpful both in classifying the different forms of  $\beta$ -thalassemia and also in the recognition of many thalassemia syndromes. In the  $\alpha$ -thalassemias, a different situation obtains (page 876).

The logical conclusion to be drawn from observations such as those described above is that thalassemia is the result of a breakdown of the mechanisms that are concerned

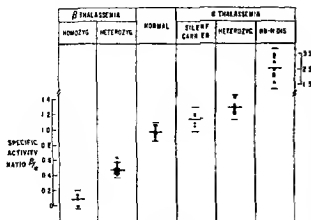


Fig 26-1. Beta/alpha synthetic ratios in various forms of thalassemia after incubation of peripheral blood with leucine- $U^{14}C$ .

The cells were incubated for two hours in autologous plasma in the presence of the labeled amino acid. The globin chains were isolated by urea-CMC chromatography. The specific radioactivity of the chains and the ratios of their radioactivity were then determined (From Nathan,<sup>14</sup> courtesy of the author and the New England Journal of Medicine.)

with normal globin-chain production.<sup>62</sup> This intricate process involves initiation of globin-chain synthesis, turning off  $\epsilon$ - and  $\gamma$ -loci and activating the  $\beta$ - and  $\delta$ -loci during the early developmental stage, synchronization of  $\alpha$ -chain synthesis with that of the other globin chains, maintaining  $\delta$ -chain synthesis at about 2.5% of that of  $\beta$ -chain production, and stopping globin production when the erythrocyte has been furnished a full complement of hemoglobin. In addition, heme synthesis must be coordinated with globin synthesis. Unfortunately, most of these activities occur in the nucleated red cell, rather than in the reticulocyte, which is the most available structure for *in vitro* studies.

Investigation has been hampered because so little is known about control mechanisms at the chromosomal level in man, as compared with models derived from microbial systems. To what extent the operon hypothesis<sup>29</sup> can account for the regulation of protein synthesis in mammalian cells is unknown, nor is the significance of the high DNA content of the cell nuclei of higher organisms in relation to protein synthesis understood.<sup>56</sup> Somewhat less obscure is the subject of globin-chain production during assembly on the polyosomes.

Sufficient has been learned about the genetic basis of thalassemia, however, to rule out a defect in heme synthesis,<sup>9</sup> or a "silent" amino acid substitution, that is, one that fails to produce an electrophoretically abnormal hemoglobin.<sup>26,27</sup> Possibilities that have not

been entirely excluded include deletion of the  $\beta$ -gene in those instances of  $\beta$ -thalassemia in which no  $\beta$ -chains are synthesized, mutations resulting in the formation of codons that dictate chain termination (eg, "amber" and "ochre"),<sup>18</sup> or the development of a mutation that would call for a different, less available transfer RNA (tRNA) to read a given codon, with resulting slowing of the rate of translation of a given messenger RNA (mRNA).<sup>28</sup> However, in most cases, neither initiation<sup>10a,19,41</sup> nor translation<sup>9,43,45</sup> has been found defective. An exception may be represented by Ferrara  $\beta$ -thalassemia<sup>153</sup> where it was found that addition of ribosome and mRNA-free supernatants from nonthalassemic subjects induced globin synthesis in thalassemic ribosomes, thereby suggesting mutation of a gene coding for a factor necessary for  $\beta$ -globin mRNA translation.

As far as most of the  $\beta$ -thalassemias are concerned, best supported is the hypothesis that the fault lies in transcription, leading to a reduced amount of normal  $\beta$  mRNA.<sup>1a,6,15,29a,41,42,46</sup> The same seems to be true of the  $\alpha$ -thalassemias.<sup>15,22</sup> Thus, mRNA isolated from reticulocytes of  $\beta$ -thalassemia directed synthesis of normal  $\alpha$ - or  $\beta$ -chains, but the amount of  $\beta$ -chains synthesized was markedly reduced. In contrast, mRNA isolated from the reticulocytes of normal blood, or sickle cell blood, directed synthesis of normal  $\alpha$ - and  $\beta$ -chains and normal  $\alpha$ - and  $\beta^S$ -chains, respectively, in normal amounts.<sup>6</sup>

The mode of inheritance of the thalassemia



disorders is consistent with the view that they are the result of autosomal (non-sex-linked) gene mutations. The genetics of the  $\alpha$ -thalassemias has been particularly difficult to fathom, as will be discussed in a later section of this chapter (page 881).

The  $\beta$ -thalassemia genes appear to be allelic or closely linked with the genes for amino-acid substitutions affecting the  $\beta$ -chains. Likewise, the  $\alpha$ -thalassemia genes are allelic or closely linked with  $\alpha$ -chain hemoglobinopathies. The  $\beta$ - and  $\alpha$ -genes occupy widely separated loci, probably on separate chromosomes (Chapter 24).

The phenomenon of interaction was mentioned above in regard to interaction between the  $\beta$ -thalassemia and Hb S genes. Similarly, interaction has been observed between  $\alpha$ -thalassemia and  $\alpha$ -structural gene mutations.<sup>268,275</sup> However, no similar interaction occurs between  $\alpha$ -thalassemia and  $\beta$ -structural gene mutations.<sup>27</sup>

### Pathologic Physiology

From the pathophysiologic standpoint, the central features of thalassemia are: (1) deficient hemoglobin synthesis resulting from deficient production of one or another of the polypeptide chains of globin and (2) accumulation of unused normal chains. The consequences are: (1) precipitation of the free polypeptide chains in the red corpuscles to form inclusion bodies, (2) red cell membrane damage, (3) shortened red cell survival, and (4) the kinetic picture of ineffective erythropoiesis. When the magnitude of the erythropoietic defect is sufficiently great, the consequences are anemia, attempted compensation by increasing and accelerating erythropoiesis, increased activity of the reticuloendothelial system (RES) because of the need to remove the products of corpuscular breakdown, and, ultimately, the accumulation in the tissues of large deposits of iron.

The most striking changes are seen in homozygous  $\beta$ -thalassemia (Cooley's anemia) and in homozygous  $\alpha$ -thalassemia. The latter results in hydrops fetalis. A review of the main findings at autopsy of patients with

Cooley's anemia provides a picture of the ultimate effects of this disease as time is allowed for their evolution.<sup>59</sup> These include evidences of anemia and of active blood formation, both medullary and extramedullary, splenomegaly, striking changes in the bones, and pigmentation of various organs resembling that of hemochromatosis.

The spleen is much enlarged and may show infarcts and adhesions. It is firm and hard. There are foci of extramedullary blood formation, with nucleated red cells, myelocytes, and megakaryocytes.<sup>66</sup> In other areas an increase of stroma and accumulations of foam cells are seen (Fig. 26-2). The foam cells give at best a faint pink color with fat stains, but they give an intense reaction with the periodic acid-Schiff (PAS) stain.<sup>47</sup> Gaucher or Gaucher-like cells have been described, in the bone marrow as well as the spleen. These have been attributed to increased catabolism of erythrocytes giving rise to increased glucocerebroside.<sup>110</sup> The Malpighian corpuscles of the spleen are approximately normal, or small.<sup>59</sup>

The bones may be greatly thickened and the long bones tend to be rectangular in shape. There is atrophy of the bone shafts and trabeculae and, at the same time, proliferation of delicate new bone. In the skull the proliferation is arranged in a more or less parallel, centrifugal pattern (Fig. 26-3).

The bone marrow is hyperplastic and contains many primitive cells, numerous nucleated red cells, and many myelocytes as well as megakaryocytes. There may be a number of macrophages, some containing small amounts of hemosiderin. Foam cells like those described in the spleen may be present in small islands. A PAS-positive substance (Chapter 1, page 29), probably unused glycogen,<sup>44</sup> has been demonstrated in the marrow normoblasts.<sup>1,21</sup>

Iron<sup>59</sup> is abundant in the parenchymal and Kupffer cells of the liver, and in lymph nodes, gastric mucosa, thyroid and adrenal glands, and is usually demonstrable in Brunner's glands, the salivary and mucous glands, the parathyroid glands, hypophysis, heart muscle, and kidney tubules. In children re-

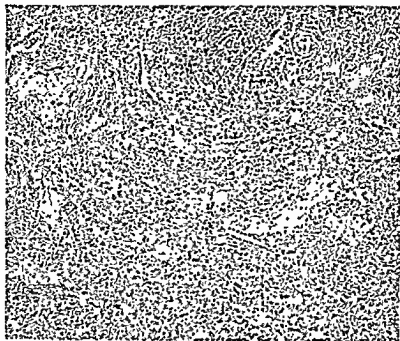


Fig. 28-2 Photomicrograph of spleen from a patient with Mediterranean anemia (thalassemia major) showing nests of foam cells in the pulp and thickened sinusoids (X200) (From Whipple and Bradford<sup>107</sup> courtesy of the authors and American Journal of Diseases of Children)

quiring regular transfusions and surviving to the ages of 8½ to 14 years, a characteristic cirrhosis, as well as myocardial degeneration and right ventricular hypertrophy, was found to be invariable and pericarditis was frequent.<sup>60</sup> The spleen shows only a moderate amount of iron within the reticuloendothelial cells and the skin contains small amounts.

It is plausible to assume that continuous marrow hyperplasia from early life causes the bone changes that are so characteristic of classical Cooley's anemia. The splenomegaly is due to extramedullary hematopoiesis<sup>66,94</sup> as well as to hyperactivity of the RES, as is the hepatomegaly. To these more specific effects can be added the consequences of chronic anemia, including impaired growth and other hormonal<sup>85,89</sup> effects. The severity of all of these changes depends on the degree of impairment in the production of hemoglobin.

The red corpuscles one sees in the blood in Cooley's anemia have the appearance of having been formed with an adequate or excessive membrane but with little hemoglobin. Their extreme thinness accounts for some of

the bizarre forms observed, including the "target" cells. Electron microscopy has shown alterations in surface texture<sup>23</sup> and gross distortion and deformity of the cells. Indentations and infolding of the plasma membrane with vacuole formation are prominent.<sup>95</sup> Intracellularly, a wide spectrum of abnormalities is found, especially in the blood of splenectomized subjects.<sup>95</sup> These include accumulations of iron in different forms within membrane-bound particles or mitochondria, and Heinz bodies in various stages of development.

Cells of patients with thalassemia major are excessively permeable to cations; cation flux is increased and there may be net loss of intracellular  $K^+$ .<sup>39,40</sup> Thalassemia minor red cells show an increase in permeability to  $K^+$ , with consequent loss of cellular  $K^+$ , but there is no change in  $Na^+$  permeability nor an increase in cellular  $Na^+$ . The increased resistance to osmotic lysis characteristic of these cells has been attributed to the water loss associated with the decreased cation content and concomitant shrinkage of the cells.<sup>23</sup>

In  $\beta$ -thalassemia, the deficit in  $\beta$ -chain synthesis results in the production of an intracellular pool of excess  $\alpha$ -chains.<sup>37,54,61</sup> The excess  $\alpha$ -chains are released into the soluble fraction of the red cell and some combine with newly made  $\beta$ - and  $\gamma$ -chains. The remainder, being unstable, rapidly precipitate and become associated with cell membrane. The rapid rate of  $\alpha$ -chain precipitation in  $\beta$ -thalassemia may be the result of hemichrome formation.<sup>44</sup> Heme has been found to be attached to some of the excess  $\alpha$ -chains in the red cells, and the excess of dipyrroles which has been described in thalassemic urine<sup>33</sup> probably reflects breakdown of excess  $\alpha$ -chains with heme attached. The excess of "early-labeled" bilirubin that accompanies the ineffective erythropoiesis<sup>14</sup> probably is derived from the intramedullary destruction of the defective red cells.

The precipitated chains form the inclusion bodies described in the marrow erythroblasts and in the circulating red corpuscles of splenectomized patients.<sup>12</sup> The alterations in membrane structure and function described above are thought to be the consequence of damage associated with inclusion body formation. These insoluble and rigid  $\alpha$ -chain precipitates probably cause fragmentation and

pitting in any area of restricted passage in the microcirculation, thereby giving rise to small, distorted cells.<sup>49</sup> Shortening of red cell survival has been found to be related directly to the degree of chain imbalance in both homozygous and heterozygous  $\beta$ -thalassemia.<sup>32,59</sup> Corpuscles containing inclusion bodies are scarce in the blood of non-splenectomized individuals, no doubt because of the pitting and scavenging activities of the spleen (Chapter 8).

The survival of red cells transfused from subjects with thalassemia major into normal individuals was found to be shortened.<sup>31</sup> It was noted that between 25 and 50% of the cells in thalassemia major blood disappeared from the recipient's circulation in 20 to 30 days, whereas the remainder followed the normal rate of elimination. The survival of red corpuscles from subjects with thalassemia minor who had no icterus was normal.<sup>7,24,31</sup>

Studies of the morphologic characteristics of bone marrow and of iron and porphyrin metabolism indicated that large quantities of heme,  $\alpha$ -chains, and red cells are produced in homozygous  $\beta$ -thalassemia.<sup>21,51</sup> Yet delivery of erythrocytes to the blood is markedly decreased<sup>11</sup> and there is quite extensive premature destruction of red cells in the marrow

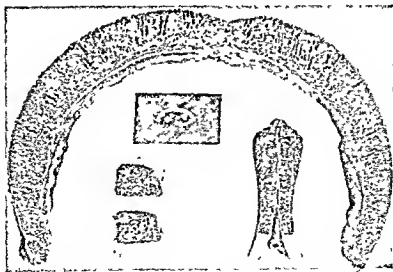


Fig 26-3. Coronal section of the calvarium, a rib and costochondral junction, vertebrae and cross section of the shaft of the femur. Case of Mediterranean anemia (From Whipple and Bradford<sup>167</sup> courtesy of the authors and American Journal of Diseases of Children)

(ineffective erythropoiesis),<sup>14</sup> as mentioned above. That there probably are two populations of red cells, one short-lived and the other with a much longer mean survival time, is suggested by the observation that the turnover rate of Hb A, as measured *in vivo* by labeling with [2-<sup>14</sup>C] glycine, is considerably higher than that of Hb F.<sup>18</sup> Differential centrifugation of blood cells revealed that the young cells contained a much higher proportion of Hb A than did older cells. The latter contained relatively more Hb F.<sup>31,39</sup> When the blood of splenectomized patients was examined it was found that the younger cells contained more inclusion bodies than did the older ones. Other differences included a lower hemoglobin content, more distortion, more rapid rate of flux of potassium across the membrane, and a higher rate of glycolysis and lactate formation with low and unstable levels of ATP in younger cells.<sup>33,31,39</sup>

To explain the increased quantities of Hb F in the red corpuscles it has been suggested that, under normal circumstances of erythropoiesis, among the early precursor erythroid cells there are cells that form  $\beta$ - or  $\gamma$ -chains or both. As these cells mature, their ribosomes lose the capacity for  $\gamma$ -chain synthesis when these cells have made only 1 to 2% of their final complement of hemoglobin. Under conditions of erythropoietic stress, erythroid cell maturation is so altered that circulating reticulocytes are derived directly from early erythroid forms that have not lost the capacity to form  $\gamma$ -chains and consequently a greater fraction of Hb F is produced.<sup>8</sup> If, in addition to the accelerated maturation, there is a defect in  $\beta$ -chain synthesis, as in many thalassemia syndromes, the result would be an even greater increase in the proportion of Hb F.

Although, as indicated earlier, a defect in heme synthesis cannot be regarded as a fundamental abnormality in thalassemia, heme synthesis is nevertheless impaired. Thus, *in vitro* studies of thalassemic red blood cells revealed defective utilization of glycine to form  $\delta$ -aminolevulinic acid (ALA). The biosynthetic step between ALA and heme appeared to be intact, but another step, that

following the formation of protoporphyrin or heme, was defective.<sup>53</sup> It was noted that thalassemic hemolysates tended to synthesize a greater percentage of protoporphyrin than did nonthalassemic erythrocytes. This suggests a block in hemoglobin synthesis following the formation of protoporphyrin or heme. It was suggested that, since ALA synthetase is the rate-limiting enzyme in the heme pathway, it is possible that, due to the defect in globin production, heme intermediates accumulate and depress ALA synthetase activity by negative feedback inhibition.<sup>3</sup> That these defects in heme synthesis may, at least in part, be explained by the excess content of iron in the tissues is suggested by the observation in one patient that enzyme activity increased following 4½ months' treatment with desferrioxamine.<sup>50</sup>

As to the manifestations of forms of thalassemia other than classical Cooley's anemia, it is plausible to presume that the remarkable heterogeneity of the thalassemias depends on the degree of chain imbalance, the relative efficiency with which free polypeptide chains are removed, and the quantity of other chains, such as  $\gamma$ -chains, with resulting production of Hb F that is produced. The degree of hypochromia associated with the various forms of thalassemia reflects the severity of the defect in globin-chain synthesis. Thus, hypochromia is not associated with  $\delta$ -thalassemia, in which only the minor  $\delta$ -chain is deficient, or with hereditary persistence of fetal hemoglobin, in which total hemoglobin synthesis is normal.

### $\beta$ - and ( $\delta\beta$ )-Thalassemias

As the result of measurements of Hb A<sub>2</sub> and Hb F, and genetic studies and measurements of the synthesis of  $\beta$ -chains, it has been shown that there are several types of genes that restrict  $\beta$ -chain synthesis and that these lead to clinical forms of thalassemia that differ in severity. The most injurious  $\beta$ -thalassemia gene ( $\beta^{\text{thal}}$ ), found especially in Mediterranean populations, results in the total absence of gene product in homozygotes. A less severe impairment of  $\beta$ -chain

synthesis is associated with a gene designated  $\beta^{\text{thal}}$ . Mildest of all is the so-called "silent" gene ( $\beta^{\text{thal}}$ ). In the heterozygous state, the "silent" gene produces no clinical, hematologic, or hemoglobin electrophoretic abnormalities, but presumably is associated with a mild deficiency of  $\beta$ -chain synthesis. Its presence is inferred when one parent of a child with thalassemia intermedia has typical  $\beta$ -thalassemia trait, whereas the other is "normal." On incubation of the normal parent's red cells with radioactive leucine, a reduced rate of  $\beta$ -chain synthesis was demonstrated.<sup>162</sup>

Still another gene (or genes) affects the synthesis of both  $\delta$ - and  $\beta$ -chains ( $(\delta\beta)^{\text{thal}}$  or  $(\delta\beta)^{\text{thal}}$ ). Finally, a family of  $\delta\beta$  "fusion genes" resulting from unequal crossing-over has been recognized; these genes produce the hemoglobin Lepore syndromes. The effects of these genes and of their combination are summarized in part in Table 26-1.

A variety of terms has been used to describe the different thalassemia syndromes which may be encountered. Unfortunately, some of these terms have been used in a way to imply genetic meanings, leading to a somewhat confused mixture of genetic and clinical terminology. The clinical terms are useful, however, for it often is impossible to define the genetic constitution of an individual with precision. Nevertheless, they should be used only as such. The severest form of  $\beta$ -thalassemia, *thalassemia major* or *Cooley's anemia*, is characterized by marked anemia from infancy, often in the range of 4 to 6 g/dl. A less severe illness has been called *thalassemia intermedia* or "mild Cooley's anemia." This implies more moderate anemia and jaundice, and the subjects may survive into adult life even without blood transfusion. *Thalassemia minor* is an asymptomatic illness, with mild or no anemia, but with prominent morphologic abnormalities of the erythrocytes. Finally, *thalassemia minima* refers to a condition which is undetectable with any certainty except by inference from genetic studies. These various forms of thalassemia will be described and related to genetic constitution in the sections that follow. Their

differentiation from one another and from other hematologic disorders will be discussed at the end of this chapter (page 883) as well as in the individual sections.

### Homozygous $\beta$ -Thalassemia

(*Cooley's Anemia*,<sup>73</sup> *Thalassemia Major*, *High A<sub>2</sub>  $\beta$ -Thalassemia*,  *$\beta$ -Thalassemia Type 1*)

### Clinical Findings

Affected infants fail to thrive. Early in infancy pallor is observed, and there may be a slight degree of icterus. The abdomen enlarges progressively, due to the increasing size of the liver and spleen. Periodic attacks of fever, diarrhea, and a history of poor feeding are common.

When the condition is fully developed, the clinical picture is characteristic. The child is small for its age but the head may appear to be large. The skin is a pale, muddy-yellow and the cheek bones are prominent. This makes the bridge of the nose appear sunken and tends to expose the upper teeth. The cranial bones are thickened ("bossing"), the eyelids may be puffy, and there may be an epicanthal fold; thus the designation "mongoloid facies." From a study of 138 patients it was concluded that the cephalofacial deformities are time related and closely reflect the severity of the illness.<sup>87</sup> Mild jaundice often is present, but this may not be apparent to the patient's family. Moderate lymphadenopathy may be found, but this is never striking. Cardiac dilatation is often present, and in advanced stages there is edema with effusion into serous cavities, and ecchymoses as well as free bleeding may develop.

### Skeletal System

Roentgenograms reveal striking changes in the skeletal system. There is thickening of the diploe of the skull to several times the normal depth. The outer and inner tables are thin, the former sometimes being invisible, and perpendicular striations appear between the

tables, resulting in an appearance suggesting hair standing erect on the scalp (Fig. 26-4). In the long bones, widening due to increase in the medullary portion, decreased density of the medulla, and thinning of the compact bone of the cortex are found, the most striking findings have been in the distal ends of the femurs. In the short bones, which are often rectangular in contour, trabeculation of the medulla, giving the bones a mosaic pattern, is the characteristic finding (Fig. 26-5).

Demonstrable abnormalities of the bone structure have been observed in patients as young as 4½ months old. The earliest lesions in the skull are in the frontal bones. The lesions in the tubular bones of the extremities regress with age, while the changes in the central segments of the skeleton, such as the skull, spine, and pelvic bones, persist and increase.<sup>60</sup> Retarded pneumatization of cranial air sinuses is common. Premature fusion of a segment of the epiphysis of the proximal end of one or both humeri or the distal end of one or both femurs is relatively common.<sup>75</sup>

Spontaneous fractures are unusual, but fractures may follow minor trauma.

Renal enlargement, due to renal tubular dilatation, has been described.<sup>78</sup>

Retarded growth is demonstrable after the patient is 4 years old and by the time he is 9 to 10 years old it often is significant.<sup>85,89</sup> However, except for diminished urinary excretion of 17-ketosteroids, endocrine function has not been found to be abnormal.<sup>85</sup> Some degree of sexual infantilism is common and secondary sexual characteristics develop late or not at all. Menarche is usually delayed or does not occur. As might be expected in chronically ill children, abnormal emotional responses, depressed mood, and floating anxiety have been observed, but no significant deviations from normal intelligence patterns have been found.<sup>85</sup>

In patients treated with frequent transfusions, pancreatic endocrine dysfunction and selective pituitary dysfunction with altered gonadotropin and thyrotropin reserve have been demonstrated in some instances.<sup>70,86</sup>

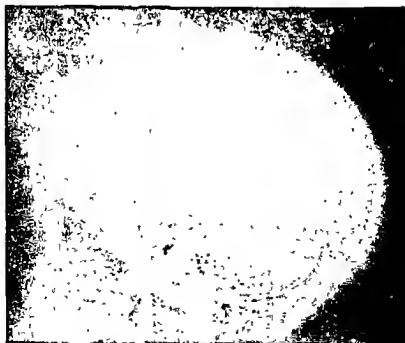


Fig 26-4. "Hair-on-end" appearance of skull produced by trabecular striations radiating outward from the inner table in a patient with thalassemia major.



Fig 26 5. Mosaic pattern produced by trabeculation in the bones of the hand, in a patient with thalassemia major. Note the rectangular contour of the metacarpals (Courtesy of Dr S J Baker)

### The Blood

The anemia is usually pronounced when it is first discovered.<sup>66,73,104</sup> The red cell count is often between  $2 \times 10^{12}$  and  $3 \times 10^{12}/l$  but it may be as high as  $4 \times 10^{12}$  or as low as  $1 \times 10^{12}/l$ . The reduction in hemoglobin (2.5 to 6.5 g/dl) and in volume of packed red cells (0.11 to 0.24 l/l) is even greater, the anemia being hypochromic (MCHC 23 to 32 g/dl RBC), microcytic (48 to 72 fl) in type.

The appearance of the red corpuscles is very characteristic (Fig. 26-6). These cells vary greatly in size, ranging from 3 to 15  $\mu m$  in diameter. They contain little pigment and may be so distorted in shape and unusual in appearance that they appear to be composed

almost exclusively of a thin, nearly colorless membrane. The hemoglobin that is present may outline only the periphery. It may also form a circular area in the center (target cell) or there may be a bridge joining the central and peripheral zones of pigment. Few fully colored cells are present.

In wet preparations the distortion of the red corpuscles is still more evident and there may be considerable fragmentation.<sup>74</sup> The edges of some corpuscles may be folded over and then the several layers may be seen to be remarkably transparent.

The presence of nucleated red cells is a characteristic finding. There may be only 10 or 20 per 100 leukocytes, or they may be several times as numerous as the white cells; in absolute terms,  $0.20$  to  $125.0 \times 10^9/l$  may



Fig 26-6 Blood smear in a case of thalassemia major. Note the hypochromia, target cells and polychromatic cells. Photomicrograph (X1050)

be found.<sup>60</sup> The majority are either typical normoblasts or microblasts, and a number of very immature normoblasts may be seen. These cells were well illustrated and described by Kato and Downey.<sup>84</sup> They are unlike the megaloblasts found in pernicious anemia (Plate IX, page 570).

In addition, other signs of active red cell regeneration, such as polychromatophilic cells, stippled erythrocytes, and occasional corpuscles with Howell-Jolly bodies, are found in the blood. Reticulocytes are increased, often to 5 or even 15%.

Although in some instances slight initial hemolysis has been noted in 0.54% saline,<sup>66</sup> *osmotic fragility* of the red corpuscles in the main is decreased. Hemolysis may not be complete in 0.2% saline and sometimes not even in water; a pale, gelatinous layer remains in the bottom of the test tubes.<sup>66,74</sup>

As already discussed, *inclusion bodies* may be found,<sup>12</sup> similar to those seen in Hb H disease (page 879) and in the unstable hemoglobin disorders (Chapter 24). These are large, usually single, have the staining prop-

erties of Heinz bodies, and develop spontaneously. They are most frequent in normoblasts and decrease in number in more mature cells, but they are "unmasked" by splenectomy. They consist to a great extent, if not exclusively, of precipitated  $\alpha$ -chains.<sup>12</sup>

As in certain other conditions associated with rapid cellular proliferation, megaloblastic anemia associated with folic acid deficiency (Chapter 14) has been observed in thalassemia major.<sup>100</sup>

The leukocytes often are increased in number, counts of  $10.0$  to  $25.0 \times 10^9/l$  being common. There may be well-marked myeloid stimulation with many immature forms, including myelocytes and myeloblasts.<sup>81</sup> As a rule, however, these forms are not numerous. Monocytes may be somewhat increased in number or, especially in infants, there may be lymphocytosis.

No significant abnormalities in the platelet count or coagulation mechanism<sup>79</sup> have been described.

The bone marrow was described earlier (page 859). Appropriate staining reveals an abundance of iron in the reticuloendothelial cells and a number of ringed sideroblasts.

*Bilirubinemia* (1 to 3 mg/dl) of the unconjugated variety is present and the icterus index is slightly or moderately increased (8 to 30). Erythrocyte protoporphyrin is increased.<sup>90</sup> The plasma copper may be increased above normal.<sup>71</sup> The serum iron is high<sup>71</sup> and serum transferrin is often totally saturated.<sup>101</sup> Serum glutamic oxaloacetic transaminase (SGOT) and lactic hydrogenase (LDH) activities usually are elevated.

### Urine

The urine often contains slightly or moderately increased quantities of urobilinogen or urobilin, and may be dark brown, due to increased excretion of dipyrroles and mesobilifuscin (Chapter 5, page 214). An occasional iron-laden, kidney epithelial cell may be found in the urinary sediment. The output of amino acids in the urine was found to be markedly increased in children with thalassemia major.<sup>72</sup>



## Kinetic Studies

Red cell survival generally ranges from 7 to 22 days, as measured by  $^{51}\text{Cr}$ -labeling.<sup>51</sup> As mentioned earlier (page 862) there are two populations of cells, a short-lived cell population containing predominantly Hb A<sub>2</sub>, and longer-surviving cells containing relatively more Hb F. Ferrokinetic studies<sup>16</sup> indicate that iron is rapidly removed from the plasma, but incorporation into red cells is markedly reduced, the iron being shunted to the RES. The plasma iron turnover rate (PITR) may be as much as ten times greater than normal, while erythrocyte iron turnover is only slightly elevated. Combined [ $^3\text{H}$ ] thymidine incorporation and microspectrophotometry indicated marked arrest of proliferation, the major fault being in the early polychromatophilic normoblast stage of maturation.<sup>96,108</sup>

## Hemoglobin Pattern

Table 26-1 lists the amounts of Hb A, Hb A<sub>2</sub>, and Hb F that may be encountered in the various genotypes of homozygous  $\beta$ -thalassemia. There are various degrees of increase in Hb F and low, normal, or elevated levels of Hb A<sub>2</sub>. Hb A may be totally absent. A trace of slowly migrating material which usually comprises less than 0.5% of the total hemoglobin has been identified as free  $\alpha$ -chain.<sup>77</sup> It seems likely that marked differ-

ences occur in the amount of Hb F synthesized in individual red cell precursors.<sup>56</sup> Some of the variability in Hb A<sub>2</sub> levels is attributable to the fact that the measurement is recorded in terms of total hemoglobin, rather than in relation to Hb A.<sup>127</sup> When calculated as A<sub>2</sub>/A<sub>1</sub>, the A<sub>2</sub> level will be increased in patients in whom Hb F levels are very high.

## Prognosis

The classical form of Cooley's anemia was recognized as being very serious and ultimately fatal, even in the first year of life.<sup>66,73</sup> Intercurrent infection was the usual cause of death, but cardiac failure, perhaps related to the heavy deposits of iron in the myocardium, was not rare.<sup>105</sup> Improved management of infections and better general health care have favorably affected prognosis. The influence of frequent transfusions and of splenectomy will be discussed below (page 867).

As already discussed, it is now recognized that more than one variety of homozygous  $\beta$ -thalassemia exists (Table 26-1) and the severity of the clinical manifestations differs in degree with corresponding differences in prognosis.

In classical Cooley's anemia, crises and remissions are unusual. The accelerated rate of erythropoiesis may lead to increased re-

Table 26-2. Manifestations of Various Clinical Forms of Thalassemia

	Major	Intermediate	Minor	Minimal
Severity of manifestations	++++	++	+, ±	±, 0
Genetics	Homozygotes	Homozygotes, double heterozygotes, rarely heterozygotes	Heterozygotes	Heterozygotes
Splenomegaly	++++	++, +++	+, 0	0
Jaundice	+++	++, +	0	0
Skeletal changes	++++, ++	+, 0	+, 0	0
Anemia (Hb.g/dl)	<7	7-10	>10	Normal
Hypochromia	++++	+++	++	+
Microcytosis	+++	++	+	0
Target cells	10-35%	++	+	±
Basophilic stippling	++	+	+	0, +
Reticulocytes (%)	5-15	3-10	2-5	1-2
Nucleated red cells	+++	+, 0	0	0

quirements for folic acid,<sup>82,100</sup> especially if dietary folate is marginal, or, in thalassemia intermedia, if pregnancy occurs.<sup>92</sup> Response to folic acid therapy has been observed to be associated with diffuse, intense bone pain and tenderness, especially in the lower extremities.

The long-term prognosis is poor because of the cardiac, hepatic, and endocrine damage resulting from the siderosis, this complication often having been aggravated by blood transfusion. In the absence of siderosis of marked degree, and if the infections which may develop are satisfactorily controlled, survival may extend into adolescence and even into adult life.<sup>17,56,146</sup>

### Treatment

Except in the severest forms of the disease, adaptation to the state of chronic anemia is remarkably good. Needless to say, treatment with iron is contraindicated, and copper, cobalt, organ extracts,<sup>55</sup> pyridoxine, and vitamin B<sub>12</sub> are of no value. Even folic acid is useless unless a relative deficiency develops. The claim that vitamin E protects some tissues from certain of the toxic effects of iron overload or has other beneficial effects requires further investigation.<sup>81</sup> Two forms of treatment deserve discussion, however, namely, blood transfusion and splenectomy. An objective, as yet only for the future, is the correction of the genetic defect by manipulation or modification of the protein-synthesizing machinery of the red cells in such a manner that the normal gene product is synthesized even though a mutation still exists in the patient's DNA.<sup>65</sup> Less ambitious, but also not yet possible, is the induction of fetal hemoglobin synthesis by arresting the switch from  $\gamma$ - to  $\beta$ -chain synthesis, or suppression of  $\alpha$ -chain synthesis, thereby to decrease  $\alpha$ -chain precipitation. As yet, bone marrow transplantation presents more problems than promise.

### Blood Transfusion

The occasional transfusion of blood has little value and should be avoided. As men-

tioned earlier, patients, especially children, with chronic anemia adapt remarkably well to even severe degrees of anemia. Any beneficial effects of transfusions are only of temporary value and yet, in time, will significantly increase the degree of hemosiderosis with all its consequences. Clearly, however, in children with severe grades of anemia, with hemoglobin levels as low as 5 or 6 g/dl or lower, blood transfusion is necessary to maintain life and normal activity. For such patients, a program of regular transfusions, in amounts sufficient to raise the hemoglobin level to 9 or 10 g/dl and maintain it there, is widely acclaimed as being worthwhile if begun early in life, probably no later than when the child is 6 years of age and preferably sooner.<sup>109</sup> To accomplish this, 400 to 900 ml whole blood, at intervals of 20 to 30 days, must be given and, at the cost of an extra iron burden of 2 to 4 g iron per year, improvement in general health and activity, decrease in heart, liver, and spleen size,<sup>94</sup> and, if undertaken before the age of 4 years,<sup>87</sup> prevention or amelioration of bone changes have been claimed.<sup>67</sup>

In patients with thalassemia of less severity, the benefits of blood transfusion must be weighed against the ill effects of the hemosiderosis which is ultimately induced, as well as the inconvenience and expense of blood transfusions given every three or four weeks. Transfusion siderosis may cause some degree of endocrine function,<sup>70,86</sup> as has been mentioned (page 864), and prepubertal growth is not improved and may be depressed.<sup>68</sup> Other risks must also be considered, such as the transmission of hepatitis. Furthermore, it is not known whether a long-continued program of regular transfusion therapy prolongs life. Most investigators would question the wisdom of instituting transfusion therapy in patients with hemoglobin levels of say 7 g/dl or higher.

Iron loading as the result of repeated blood transfusions can be controlled in some degree by the use of an iron chelator, desferrioxamine (DF)<sup>103</sup> being preferable to diethylenetriaminepentaacetic acid (DTPA).<sup>91</sup> The average intramuscular dose of DF will cause the urinary excretion of approximately 10 mg of

iron. Since 250 ml packed red cells contribute about 250 mg of iron, if this amount of blood is given every 20 to 30 days, daily injections of DF would have to be given to prevent progressive accumulation of iron. This is rather impractical and, with most transfusion regimens, less DF is given. Clearly, better iron chelators are needed. It has been reported that daily oral administration of 200 mg ascorbic acid increases the response to DF.<sup>93</sup>

### Splenectomy

Splenectomy has been advocated<sup>102</sup> when anemia has begun to develop more rapidly following blood transfusion than previously, the implication being that the accelerated red cell destruction is due to an extracorporeal mechanism. The spleen also has been removed when thrombocytopenia of severe degree has developed,<sup>182a</sup> presumably due to splenic sequestration, or when the size of the spleen has become so great as to cause extreme discomfort.<sup>97,105</sup> However, especially in children under 4 years of age, the risk from the development of sudden, overwhelming infection appears to be much greater following splenectomy than in older children; in thalassemia major, this risk seems to be much higher than following splenectomy for more benign conditions.<sup>76</sup> The pneumococcus has been the most frequent organism involved, and for this reason daily prophylactic administration of penicillin or sulfisoxazole has been recommended for a younger child for a year or two after splenectomy. Raised anti-streptolysin titers have also been reported and it has been suggested that benign pericarditis occurring post-splenectomy is attributable to such streptococcal infections.<sup>106</sup> Serum immunoglobulin levels for IgG and IgA have been found to be increased after splenectomy<sup>101,106</sup> but whether IgM is increased<sup>101</sup> or reduced<sup>106</sup> is unsettled.

The decision whether a given patient should be treated by transfusions, a splenectomy, or both, or if neither method should be employed is a most difficult one. Both procedures carry risks; the potential benefits must be weighed in each case, and reeval-

uated from time to time in the same case. There is a paucity of conclusive data on which the decision can be based.

### Complications

Complications, such as infections, must be met as they arise. Epistaxis is not uncommon, but is unrelated to any abnormality in coagulation or thrombocytopenia. Severe facial deformity has been corrected surgically.<sup>83</sup>

### Thalassemia Intermedia

As has been stated, thalassemia intermedia refers to cases in which the clinical manifestations are intermediate in severity between Cooley's anemia (thalassemia major) and thalassemia trait (thalassemia minor) and should be used only in a clinical sense. It has been used to apply to less serious forms of the homozygous disease,<sup>113,114,117,120,132</sup> but it has also been applied to patients who seem to be heterozygous<sup>116</sup> (Table 26-1). Some patients with clinically benign cases of thalassemia major may be doubly heterozygous for two different types of retardation of  $\beta$ -chain synthesis,<sup>120,128,137</sup> eg, heterozygosity for  $\beta$ -thalassemia and ( $\delta\beta$ )-thalassemia,  $\beta$ -thalassemia and a structural hemoglobin variant, or  $\beta$ -thalassemia and Hb Lepore.

The degree of globin precipitation in the marrow of patients with thalassemia intermedia has been found to be much milder than in patients with classical cases of Cooley's anemia<sup>61</sup>; this probably is an important factor influencing the severity of the clinical manifestations. In two reported families, inheritance of both  $\alpha$ - and  $\beta$ -thalassemia produced a clinical syndrome of intermediate severity. It was postulated that, with fewer excess  $\alpha$ -chains available than in homozygous  $\beta$ -thalassemia, intramedullary and peripheral hemolysis were less pronounced.<sup>123</sup>

Patients with thalassemia intermedia may survive into adult life,<sup>118</sup> and even enjoy a full life span.<sup>137</sup> Chronic anemia of various degrees is present, and other manifestations such as splenomegaly, bone changes, and the effects of the accumulation of iron in the tissues vary.<sup>116</sup> A high incidence of masses

of extramedullary hematopoiesis, demonstrable by roentgenography,<sup>123</sup> and of cholelithiasis<sup>115</sup> has been described.

It has not been possible to prove the heterozygous inheritance of  $\beta$ -thalassemia intermedia, some claims to the contrary notwithstanding.<sup>113,131</sup> One parent of a patient with thalassemia intermedia who seemed to be normal may instead have been a carrier of the silent  $\beta$ -thalassemia gene, while the other parent had typical thalassemia trait.<sup>111,112,110</sup> In many of the reported families the propositus has been a patient with high levels of Hb F (20 to 50%) and increased levels of Hb A<sub>2</sub>.<sup>112</sup>

### Heterozygous Beta Thalassemia

#### Clinical Manifestations

The heterozygous state for  $\beta$ -thalassemia not infrequently is discovered accidentally, either during examination for unrelated symptoms, or because hypochromia, target cells, or basophilic stippling was noted in a few red corpuscles in the blood smear<sup>146</sup>; in the investigation of a moderate anemia or a palpable spleen; or in a family study. In anemic patients, often a mistaken diagnosis of iron-deficiency anemia has been made and the patients treated for this condition. The clinical picture can range from that of a patient with thalassemia intermedia, ie, chronic anemia and splenomegaly, sometimes with attacks of acute abdominal pain, hemosiderosis,<sup>145</sup> and even with skeletal changes suggestive of the homozygous state,<sup>56</sup> to the more common more benign state (*thalassemia minor*) associated with mild or no anemia, perhaps complicated by leg ulcer<sup>136</sup> or gallstones (Rietti-Greppi-Micheli syndrome, Chapter 24); or the condition may be almost entirely without recognizable features (*thalassemia minima*, *microcytemia*<sup>140</sup>) (Table 26-2). Thinning of the cortex of the long bones and osteoporosis may be noted (Fig. 26-7)<sup>138</sup> and even radiologically demonstrable tumors of extramedullary erythropoiesis may be present.<sup>126</sup> Several genotypes are listed in Table 26-1).



Fig 26-7. Femur of a boy, aged 18 years who had thalassemia minor (A), compared with that of a healthy boy of the same age (B). Note the thinness of the cortex and the generally decreased density in A.

#### Laboratory Findings

##### Blood Findings

The red cell count may be moderately elevated above normal, but MCH is reduced, as is MCV, while MCHC may be normal or only slightly reduced. In a series of 45 cases hemoglobin was  $11.6 \pm 1.04$  g/dl, MCV  $64.70 \pm 4.35$  fl (range 52 to 75), MCH  $20.26 \pm 2.23$  pg, and MCHC  $31.22 \pm 0.96$  g/dl RBC.<sup>135</sup> Because the typical erythrocytes are flat (leptocytic), they may appear normal in diameter and hypochromic even though the indices suggest that they are

microcytic and normochromic. Other findings on microscopic examination include aniso- and poikilocytosis, basophilic stippling, and target cells. These may be very striking (Fig. 26-8) and may be far out of proportion to the degree of anemia. Resistance to hemolysis by hypotonic saline solutions is strikingly increased. On the other hand, as noted previously, no red cell changes may be demonstrable (eg, in  $\beta^{thal}$ , Table 26-1).

As in thalassemia major, the plasma copper may be increased above normal, the free erythrocyte protoporphyrin may be increased<sup>11</sup> (although this is not always the case<sup>21,141</sup>) and the iron-binding capacity of the serum may be completely saturated. (See Chapter 16, page 627).

Red cell survival time may be normal or slightly shortened.<sup>31,130,134</sup> There may be modest evidence of ineffective erythropoiesis.<sup>134</sup> If a splenectomy has been performed,  $\alpha$ -chain inclusions may be found in the red corpuscles in the blood.<sup>61</sup>

### Hemoglobin Pattern

The most consistent feature of the hemoglobin pattern of thalassemia minor is an increase in the proportion of Hb A<sub>2</sub>. As compared with a value of  $2.54 \pm 0.35\%$  in 300 normal individuals, the mean level in 34 thalassemia carriers was  $5.11 \pm 1.35\%$ .<sup>127</sup> With the exception of the carriers of some unstable hemoglobins (Chapter 24) and Hb C and Hb S heterozygotes and homozygotes,<sup>80</sup> an increased level of Hb A<sub>2</sub> has not been found in any other hemoglobin abnormality.<sup>56</sup> This useful diagnostic feature is lost when there is iron deficiency, but is corrected after iron therapy.<sup>124,143</sup> The increase in Hb A<sub>2</sub> described in some patients with pernicious anemia in relapse should cause no confusion in diagnosis.<sup>121</sup>

It is not known whether the Hb A<sub>2</sub> is homogeneously distributed throughout the red cells. If it is, the absolute amount per cell can be calculated from the MCH. When this was done, the Hb A<sub>2</sub> content of the red corpuscles was about 1.0 pg as compared with

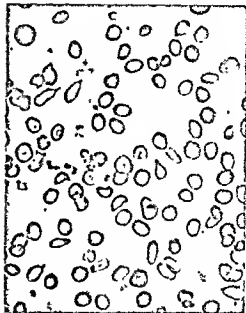


Fig. 26-8. Stained smear showing the marked hypochromia, moderate poikilocytosis, and microcytosis, as well as a few target cells in an 18 year old boy with thalassemia minor. Photomicrograph (X800)

a normal value of 0.75 pg.<sup>56</sup> The increased synthesis of Hb A<sub>2</sub> probably is due to increased activity at both  $\delta$ -chain loci. Intra-familial segregation of Hb A<sub>2</sub> values in thalassemia minor has been noted.<sup>119</sup>

Hb F either is not detectable at all or, with rare exceptions, is present in extremely small amounts.<sup>144</sup> The distribution of the fetal hemoglobin within the red cells is heterogeneous.<sup>139</sup>

### ( $\delta\beta$ )-Thalassemia

#### (F-Thalassemia, Normal Hb A<sub>2</sub> $\beta$ -Thalassemia, $\beta$ -Thalassemia Type 2)

This form of thalassemia<sup>171</sup> is the result of defective  $\delta$ - and  $\beta$ -chain synthesis. As a consequence, in the homozygous condition<sup>151,161,163</sup> the hemoglobin is entirely fetal in type ("F-thalassemia") and Hb A and Hb A<sub>2</sub> are completely absent. The clinical manifestations have been those of thalassemia intermedia and many of the reported patients have been adults. Hemoglobin levels usually have ranged from 9 to 12 g/dl.

Heterozygotes<sup>144,154,156,157,159,163,165,168,171</sup> have Hb A, Hb A<sub>2</sub>, and Hb F. The Hb A<sub>2</sub> level usually is normal but, if expressed per red cell, the absolute mean value is about 0.53 pg (normal 0.75 pg) since MCH is reduced.<sup>165</sup> Hb F values have been 5 to 20% and averaged 10.9%. There are no specific clinical features and the red cell changes are indistinguishable from those of  $\beta$ -thalassemia minor.

( $\delta\beta$ )-Thalassemia may be associated with  $\beta$ -thalassemia ("heterozygosity for ( $\delta\beta$ )- and  $\beta$ -thalassemia"<sup>154,157,165,169,170,171</sup>). The clinical features have been found to be extremely variable, ranging from anemia, jaundice, splenomegaly, and bone changes in childhood to chance discovery later in life. Hemoglobin levels have ranged from 5.3 to 13.1 g/dl. Fetal hemoglobin is in the 60 to 95% range or higher,<sup>161</sup> Hb A<sub>2</sub> usually is 1 to 2% and Hb A may or may not be demonstrable.<sup>165</sup>

Analysis of the fetal hemoglobin in a Chinese family showed that all the individuals carrying the ( $\delta\beta$ )-thalassemia gene synthesized only the G<sub>7</sub> variety<sup>159</sup> (see page 876). This differs from findings in other patients with ( $\delta\beta$ )-thalassemia.<sup>163</sup> These observations provide evidence for the heterogeneity of this form of thalassemia.

### Miscellaneous Varieties

A number of other forms of  $\beta$ - and ( $\delta\beta$ )-thalassemia have been described. Heterozygosity for ( $\delta\beta$ )-thalassemia and the  $\delta$ -chain variant, Hb A'<sub>2</sub> (or B<sub>2</sub>) was associated with the presence of hemoglobins A, F, and B<sub>2</sub>, the last amounting to 3%.<sup>152</sup> However, since Hb A<sub>2</sub> was completely absent, it would appear that the ( $\delta\beta$ )-thalassemia mutation completely suppressed the *cis*- $\delta$  gene and that the remaining normal  $\delta$ -gene was capable of increasing  $\delta$ -chain synthesis sufficiently to bring the Hb A'<sub>2</sub> (B<sub>2</sub>) to within normal range.

$\beta$ -Thalassemia ("A<sub>2</sub> F-thalassemia") with unusually high levels of Hb F in heterozygotes, 5.1 to 14.4%, and even 17%,<sup>144</sup> in contrast to the usual low or at most 5% level characteristic of thalassemia minor (page 871) has been reported.<sup>137</sup> Hb A<sub>2</sub> values were

higher (6.6%) than usually found in  $\beta$ -thalassemia heterozygotes. In the homozygous state, this variety of thalassemia produced a mild hemolytic state (thalassemia intermedia).

Deficient synthesis of  $\gamma$ - and  $\beta$ -chains in relation to  $\alpha$ -chain synthesis ([ $\gamma\beta$ ]-thalassemia) produced hemolytic hypochromic anemia at birth which became modified as the infant matured to attain the findings of  $\beta$ -thalassemia in her father and six other relatives.<sup>158</sup>

The presence of inclusions in normoblasts consisting of precipitated  $\alpha$ -chains characterized a form of heterozygous  $\beta$ -thalassemia of greater than usual severity observed in a family of Swiss-French descent.<sup>164</sup> A form of severe  $\beta$ -thalassemia which was thought to result from overproduction of  $\alpha$ -chains also has been described.<sup>55</sup>

### $\delta$ -Thalassemia

An inherited defect in  $\delta$ -chain synthesis, occurring in an individual thought to be doubly heterozygous for this condition and ( $\delta\beta$ )-thalassemia,<sup>155</sup> as well as double heterozygosity for  $\delta$ -thalassemia and hereditary persistence of Hb F, homozygous S disease and  $\delta$ -thalassemia,<sup>167</sup> and homozygosity for  $\delta$ -thalassemia,<sup>160</sup> has been described. None of the reported patients has had any clinical symptoms or hematologic changes. The importance of the recognition of  $\delta$ -thalassemia is in the fact that it explains the absence of Hb A<sub>2</sub> in certain individuals, thereby masking the presence of  $\beta$ -thalassemia, or the finding of lower levels than would otherwise be expected.<sup>56</sup>

### Association of $\beta$ -Thalassemia with the Hemoglobinopathies

#### Hb S—Sickle Cell-Thalassemia—Microdrepanocytic Disease

The earliest instances of "sickle cell anemia in white persons" were observed in persons of Greek or Italian ancestry. It was ultimately shown that these cases represented double

heterozygosity for Hb S and  $\beta$ -thalassemia.<sup>182,185</sup> Since then the condition has been found in many racial groups.<sup>56</sup> The clinical findings range from those of a disorder indistinguishable from sickle cell anemia to a practically symptom-free state. Often fewer sickle cells and more target cells are found than are usual in sickle cell anemia. The prognosis in general corresponds to the severity of the clinical manifestations. The electrophoretic pattern usually shows a preponderance of Hb S while Hb A may be as low as 5 to 10%,<sup>181</sup> or even absent<sup>184</sup>; in the form associated with the  $\beta^{\text{thal}}$  gene there is no Hb A (Table 26-1). Hb A<sub>2</sub> usually is increased. Hb F levels range from 2 to 30%. Studies of *in vitro* hemoglobin synthesis showed that an excess of  $\alpha$ -chains is produced, with resulting ineffective erythropoiesis.<sup>56</sup> In asymptomatic black subjects, however,  $\alpha$ - and  $\beta$ -type chain synthesis in bone marrow cells was balanced.<sup>175</sup>

The genetic pattern in most cases of sickle cell-thalassemia has suggested that there is allelism or close linkage between the  $\beta$ -thalassemia and the  $\beta^S$ -loci. The combination of ( $\delta\beta$ )-thalassemia with Hb S also has been described.<sup>186</sup> In these subjects, Hb A also was completely absent, the main components being Hb F and Hb S.

In addition to the above-mentioned forms of "interacting" (page 857) sickle cell-thalassemia, a non-interacting variety was described in which there was no reversal in the hemoglobin A/S ratio and the levels of Hb A<sub>2</sub> were normal or low.<sup>178</sup> The hypothesis that such cases represent double heterozygosity of  $\alpha$ -thalassemia and Hb S<sup>27</sup> is strengthened by the description of additional syndromes involving the association of  $\alpha$ -thalassemia and  $\beta$ -chain variants (page 881).

### Hb C-Thalassemia

Like Hb S-thalassemia, Hb C-thalassemia has most often been of the interacting form, the level of Hb C exceeding that of Hb A.<sup>56</sup> The reverse<sup>191</sup> is unusual. In the Negro the disorder has been symptomless, but in the reported Italian and Turkish patients variable

anemia and splenomegaly were present. The appearance of the blood smear is very similar to that of homozygous C disease (Chapter 24).

A Negro family with Hb S, Hb C, and thalassemia in various combinations has been described.<sup>180</sup>

### Hb E-Thalassemia

Hb E-thalassemia was first described in Thailand<sup>168,177</sup> and has been observed in individuals from a number of Asiatic countries.<sup>176,189</sup> The clinical picture resembles that of Cooley's anemia. The usual electrophoretic pattern reveals Hbs E and F with no detectable Hb A. The Hb F levels show wide variations.

### Other Combinations

$\beta$ -Thalassemia has been found in association with Hb D<sup>179,187</sup> and Hb G,<sup>183</sup> as well as Hb J<sup>Georgias</sup>, Hb J<sup>Baltimore</sup>, and several  $\alpha$ -chain structural variants,<sup>56</sup> and also in association with hereditary elliptocytosis.<sup>173</sup> Homozygous Hb S disease- $\delta$ -thalassemia has been observed.<sup>185</sup>

### The Hemoglobin Lepore Syndromes

In the course of a study of thalassemia in a family named Lepore, a new hemoglobin variant was discovered.<sup>208</sup> This was later shown to be composed of normal  $\alpha$ -chains combined with chains consisting of the N-terminal residues of the  $\delta$ -chain and the C-terminal residues of the  $\beta$ -chain.<sup>200</sup> In this original Lepore hemoglobin, now called Hb Lepore Washington (=Boston), the change-over from the  $\delta$ -like sequence to the  $\beta$ -like sequence has been shown to occur between positions 87 and 116 of the new chain, which, like normal  $\delta$ - and  $\beta$ -chains, is 146 residues long. Since the original discovery, two other Lepore hemoglobins have been found, Hb Lepore Hollandia<sup>202,204</sup> and Hb Lepore Baltimore,<sup>215</sup> which are unlike Hb Lepore Washington in that the positions of fusion differ, occurring between positions 22 and 50 in the

former and between residues  $\delta^{50}$  and  $\beta^{86}$  in the latter. It has not been possible to make a closer estimate of the position of fusion of the two chains because the amino acid sequences of the  $\beta$ - and  $\delta$ -chains are so similar. Hb Pylos,<sup>207</sup> Hb Lepore the Bronx,<sup>219</sup> and Hb Lepore Augusta<sup>211</sup> have been shown to be the same as Hb Lepore Washington.

### Genetic Mechanism<sup>212</sup>

Chemical studies of the Lepore hemoglobins are consistent with the interpretation that the part of the structural gene corresponding to the N-terminal end of the  $\delta$ -chain has fused to that part of the  $\beta$ -chain which corresponds to the C-terminal end of the  $\beta$ -chain (Fig. 26-9). This is possible because the two loci are on the same chromosome and probably are closely linked. It was suggested that during meiosis the  $\beta$ -locus on one chromosome has paired aberrantly with the  $\delta$ -locus on its partner chromosome.<sup>200</sup> If crossing-over occurs, a new gene would be formed which would direct the synthesis of a peptide chain with the  $\delta$ -sequence at one end and the  $\beta$ -sequence at the other.

If this hypothesis is correct, one would expect the formation of a gene which directs the N-terminal sequence of the  $\beta$ -chain and the C-terminal segment of the  $\delta$ -chain, an "anti-Lepore" gene. Three hemoglobins have been reported which have properties that might be expected of such an anti-Lepore type of hemoglobin, Hb Miyada<sup>223</sup>, Hb P Congo<sup>205,213</sup> and Hb P-Nilotic.<sup>174</sup> No clinical

evidence of thalassemia was present in these subjects, presumably because of the presence of an active  $\delta$ - and  $\beta$ -chain gene on the same chromosome as the ( $\delta\beta$ )-fusion gene, as shown in Figure 26-9.

### Clinical Manifestations

The clinical manifestations of the Lepore syndromes are similar to those of  $\beta$ -thalassemia. Heterozygous carriers, double heterozygosity for Hb Lepore and  $\beta$ -thalassemia,<sup>203,207,208</sup> ( $\delta\beta$ )-thalassemia,<sup>223</sup> Hb S,<sup>221,222</sup> Hb C,<sup>219</sup> and Hb Peterborough,<sup>210</sup> as well as homozygous individuals<sup>207,214</sup> have been described among Italians,<sup>171,201,209,212,217</sup> Greeks,<sup>207</sup> American Negroes,<sup>215,219</sup> Yugoslavs,<sup>206</sup> Turkish Cypriots,<sup>203</sup> Papuans,<sup>214</sup> Indians and Jamaicans.<sup>66</sup> In heterozygotes, slight splenomegaly may be found,<sup>207</sup> as well as slight hypochromia and other features characteristic of thalassemia minor, including slight shortening of red cell survival.<sup>216</sup> The composition of the circulating hemoglobin has been found to include Hb A, Hb F (1.3 to 14%), Hb Lepore (6 to 15%) and Hb A<sub>2</sub> (1.2 to 2.6%).<sup>221</sup> Hemoglobin electrophoresis at an alkaline pH shows the presence of approximately 10% of a slowly migrating hemoglobin, Hb Lepore. The electrophoretic mobility is identical with that of Hb S, Hb D, and Hb P (Fig. 24-2, page 807).

The homozygous state resembles that of Cooley's anemia, with severe anemia existing from childhood. The hemoglobin has con-

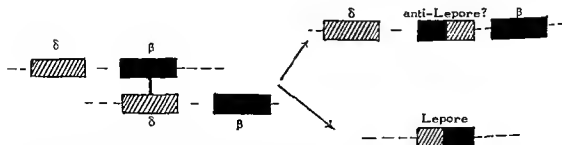


Fig. 26-9 Creation of a fusion gene through crossing-over within a gene, as is thought to have occurred to produce the gene for Lepore hemoglobin, and possibly also an "anti-Lepore" gene



sisted of approximately 75% Hb F, the remainder being Hb Lepore. No Hb A or Hb A<sub>2</sub> has been found. Double heterozygosity of Hb Lepore and  $\beta$ -thalassemia is likewise associated with anemia dating from early life, and with mongoloid facies and hepatosplenomegaly.<sup>218,220</sup> The picture differed in Italians and Greeks, however, since Hb A (20 to 40%) and Hb A<sub>2</sub> (1.2 to 3.0%) were found in Italian patients<sup>169,217</sup> and neither could be demonstrated in the Greek subjects.<sup>207</sup> This difference could be due to the type of  $\beta$ -thalassemia gene associated with the Lepore hemoglobin,  $\beta^{\text{thal}}$  in the Greek subjects,  $\beta^{\text{thal}}$  in the Italians.

Studies of the relative rates of synthesis of the constituent globin chains in reticulocytes of individuals with the Lepore trait showed that Hb Lepore is not preferentially destroyed. The specific activities of the  $\alpha$ - and  $\beta$ -chains of Hb A and the  $\alpha$ - and  $\delta\beta$ -chains of Hb Lepore were similar.<sup>224</sup> It is likely that Hb Lepore is synthesized at a low rate, lower than that for the  $\delta$ -chains of Hb A<sub>2</sub> and the  $\beta$ -chains of Hb A.<sup>56</sup>

### Hereditary Persistence of Fetal Hemoglobin (HPFH)

Not to be confused with F or ( $\delta\beta$ )-thalassemia (page 871) is a benign condition characterized by the persistence of fetal hemoglobin synthesis into adult life.<sup>234</sup> In contrast to the uneven distribution of Hb F in conditions in which Hb F is produced in partial compensation for defective  $\beta$ -chain synthesis, in HPFH the fetal hemoglobin is distributed uniformly among the erythrocytes.<sup>252</sup>

### Varieties of HPFH

First observed in Negro populations,<sup>233,234</sup> HPFH was later recognized in Greeks.<sup>235</sup> The two types differ from one another in that the percentage of Hb F in the Negro heterozygote is almost twice as high as that in the Greek heterozygote, and hemoglobin A<sub>2</sub> is lower in the Negro than in the Greek form, and some clinical differences are present as

well (see below). Cases described in racial groups other than Negro or Greek have mostly resembled the Negro type,<sup>230,254,256</sup> but in others the proportions of hemoglobins F and A<sub>2</sub> were intermediate between these two forms.

The pattern of inheritance of the Greek type is like that seen in Negroes, namely, a single autosomal co-dominant factor which is closely linked or allelic to the gene determining the structure of the  $\beta$ -chain or the  $\beta$ -thalassemia gene.

The "Swiss variety" of HPFH<sup>246,249</sup> probably is a fundamentally different entity. In this condition 1 to 3% Hb F is synthesized throughout life and Hb F is not uniformly distributed among the red cells.<sup>56</sup>

### Clinical Manifestations

The heterozygous state of the Negro type is not associated with clinical or hematologic abnormalities. Hb F has ranged from 17 to 38% and was identical in chemical structure to normal fetal hemoglobin.<sup>233,252,255</sup> Hb A<sub>2</sub> is somewhat low (1.6 to 2.2%).<sup>233</sup> The homozygous state<sup>259</sup> may be associated with a very mild polycythemia.<sup>232,253</sup> In one individual, who also had elliptocytosis, the reticulocyte count was slightly raised.<sup>250</sup> In the homozygotes, Hb A and Hb A<sub>2</sub> were completely lacking and the hemoglobin was 100% Hb F. Oxygen affinity was high in the ooe patient so examined,<sup>232</sup> as it is in fetal red cells.

The Negro type has been encountered in association with Hb S.<sup>233,234,236,242,245,255,258</sup> In patients homozygous for Hb S who also inherit a gene for HPFH, there usually are neither symptoms of anemia nor complications, with the rare exception of aseptic necrosis of the femoral head.<sup>233,242</sup> The relatively mild nature of the illness may be explained by the presence of Hb F in all the red cells in HPFH. It has been shown that in sickle cell anemia the cells containing the lowest levels of Hb F are destroyed prematurely.<sup>231</sup>

Other combinations have been described, eg, with Hb C.<sup>233,244,245</sup> In such cases, symp-

toms were lacking, anemia was absent or slight, but the red cells showed moderate variations in size and shape with many target cells. Double heterozygosity with  $\beta$ -thalassemia<sup>233,244,249</sup> is similar clinically to thalassemia minor or minima.

In the heterozygous state of the Greek form no clinical or hematologic abnormalities have been described.<sup>235</sup> In double heterozygotes with  $\beta$ -thalassemia, however, slight anemia (Hb 8 to 11 g/dl), icterus, and splenomegaly were found, microcytosis and hypochromia (MCH 20 to 23 pg) were present; and Hb F ranged from 26 to 43% and Hb A<sub>2</sub> from 3.8 to 5.2%. Greek-type homozygous HPFH has not been reported.

### Chemical and Molecular Considerations

In normal newborns, fetal hemoglobin is a mixture of two molecular species,<sup>251</sup>  $\alpha_2\gamma_2^{136\text{Gy}}$  (G<sub>γ</sub>) and  $\alpha_2\gamma_2^{136\text{Ala}}$  (A<sub>γ</sub>). The ratio of these two species in the normal newborn is 3:1. In Negro families with HPFH,<sup>241</sup> one group was found in which only a single type of structural  $\gamma$ -chain gene, G<sub>γ</sub>, was present. In a second group, only A<sub>γ</sub>-chains were found.<sup>238</sup> In the majority of the families studied, however, both A<sub>γ</sub>- and G<sub>γ</sub>-chains were formed, usually in the adult G<sub>γ</sub>/A<sub>γ</sub> ratio of 3:2. The ratios of Hb<sub>A<sub>γ</sub></sub> and Hb<sub>B<sub>γ</sub></sub> are not the same in all individuals with this form of HPFH, as exemplified by observations in four Indian families.<sup>251a</sup> In Greek HPFH heterozygotes, only A<sub>γ</sub>-chains were synthesized.<sup>239,240</sup>

HPFH is considered to be a form of  $\beta$ -thalassemia, in the sense that it is characterized by deficient  $\beta$ -chain synthesis. In the Negro type,  $\beta$ - and  $\delta$ -chain synthesis are completely lacking in the  $\alpha\text{S}$  position, whereas in the Greek form the deficit is only partial. Synthesis of  $\gamma$ -chain compensates for these deficits. Several genetic explanations have been proposed, including abnormalities in operator or regulator genes<sup>248</sup> or deletion of the  $\beta$ - and  $\delta$ -chain genes with persistent  $\gamma$ -chain synthesis in one or more of the  $\alpha\text{S}$   $\gamma$ -loci.<sup>56,239,241</sup>

### Differential Diagnosis of HPFH

The acid elution technique<sup>243</sup> for fetal hemoglobin determination provides an essential test in the differential diagnosis of HPFH because it permits one to see whether or not Hb F is more or less homogeneously distributed among the red cells (Fig. 26-10). In no other condition is such a uniform pattern of distribution found.

$\beta$ -Thalassemia minor of the type associated with unusually high levels of Hb F<sup>137,144</sup> (page 871) could be confused with HPFH but is distinguished by the high Hb A<sub>2</sub> level. However, in heterozygous ( $\delta\beta$ )-thalassemia, levels of Hb A<sub>2</sub> are not increased and confusion is possible, but the level of Hb F tends to be lower than in HPFH, distribution of Hb F in the red cells is heterogeneous, and MCH is low. Clinical and family studies, the presence of some evidence of hemolytic anemia, the morphologic appearance of the red corpuscles, and lower levels of Hb F distinguish sickle cell disease and sickle cell-thalassemia, as well as Hb C-thalassemia, from heterozygosity for both HPFH and Hb S, or for HPFH and Hb C, respectively. In addition, homogeneity of Hb F distribution in the red cells distinguishes not only the above conditions from HPFH but also from other diseases, such as aplastic anemia, in which Hb F may be increased in the presence of a normal level of Hb A<sub>2</sub>.<sup>252</sup>

### The $\alpha$ -Thalassemias

That deficiency of  $\alpha$ -chain production can occur was learned indirectly, first through the discovery of Hb H ( $\beta_4$ ) disease<sup>253,323</sup> and then through the characterization of Bart's hemoglobin ( $\gamma_4$ ).<sup>205</sup> The clinical manifestations of these conditions were those of thalassemia, but the hemoglobins were shown to be tetramers of normal  $\beta$ - and  $\gamma$ -chains, respectively, formed because  $\alpha$ -chain production was deficient.

#### Heterozygous $\alpha$ -Thalassemia

Characterization of  $\alpha$ -thalassemia has proved difficult because the hematologic

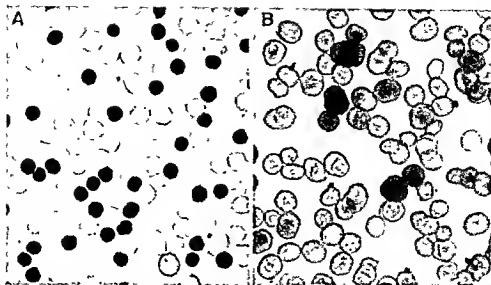


Fig. 26-10. Distribution of fetal hemoglobin in red cells as demonstrated by method of Kleihauer and Betke. A, Red cells from a normal adult mixed with cells from a homozygote of HPFH. Two distinct populations are seen: the darkly stained cells contain only Hb F, the decolorized cells are the ghosts of normal erythrocytes after elution of Hb A. B, Red cells from an adult heterozygote for HPFH, there is a fairly uniform distribution of Hb F. (From Shepard et al.,<sup>139</sup> courtesy of the authors and Bulletin of the Johns Hopkins Hospital.)

changes in heterozygous  $\alpha$ -thalassemia are extremely mild. Furthermore, because a defect in  $\alpha$ -chain production affects equally the synthesis of hemoglobins A,  $A_2$ , and F, the helpful clues suggesting the presence of  $\beta$ -thalassemia that are derived from measurement of levels of these hemoglobins are lacking. Measurement of  $\alpha$ - and  $\beta$ -chain synthetic rates in reticulocytes of relatives of patients with Hb H disease and the hydrops fetalis syndrome<sup>326</sup> reveal reduced  $\alpha/\beta$  synthetic ratios. These studies support<sup>351</sup> the assumption that, when available, measurement of the level of Hb Bart's in newborn infants is a good means of detecting deficiency of  $\alpha$ -chain production,<sup>309,318,383</sup> and its degree, although this may not be invariably true.<sup>300</sup> Based on such information, as well as on measurements of hematologic parameters,<sup>318</sup>  $\alpha$ -thalassemia carriers have been divided into two groups, those with detectable hematologic changes (mild anemia, low MCV and MCH, decreased osmotic fragility, Hb  $A_2$  reduced), designated  $\alpha$ -thalassemia<sub>1</sub> carriers, and those with normal hemoglobin and red cell osmotic fragility but some reduction in

MCV and MCH, designated "silent carriers" or  $\alpha$ -thalassemia<sub>2</sub>. Thus, in one series of children one to six years of age who had had 5 to 6% Hb Bart's at birth, the hemoglobin was  $11.0 \pm 2.3$  g/dl; MCV,  $71.7 \pm 4.4$  fl; MCH,  $21.7 \pm 1.7$  pg; and MCHC,  $30.2 \pm 0.28$  g/dl. In children who had had 1 to 3% Hb Bart's at birth these values were more nearly normal (Hb,  $11.7 \pm 0.65$  g/dl; MCV,  $77.7 \pm 3.5$  fl; MCH,  $25.6 \pm 2.1$  pg; MCHC,  $32.5 \pm 2.0$  g/dl).<sup>351</sup> In another report of blood findings in  $\alpha$ -thalassemia carriers of unstated age, hemoglobin was  $12.66 \pm 1.14$  g/dl; MCV,  $72.21 \pm 3.33$  fl (range 63 to 78 fl); MCH,  $23.22 \pm 1.33$  pg; and MCHC  $31.87 \pm 0.87$  g/dl.<sup>135</sup>

Clinical disorders due to deficiency of  $\alpha$ -chain production result from the interaction of (at least) these two genes<sup>339,341</sup> (Table 26-3). The clinical manifestations of heterozygous  $\alpha$ -thalassemia are similar to those of  $\beta$ -thalassemia minor. The diagnosis is suspected when the typical morphologic characteristics are detected, especially if familial transmission can be demonstrated and iron deficiency can be excluded, but there are no

Table 26-3. Classification of the  $\alpha$ -Thalassemias

Genotype	Clinical Severity	Hb Bart's in Cord Blood	Hb H in Adults (%)	Other
$\alpha\alpha$	Normal	<0.5%	0	—
$\alpha\alpha^{thal_1}$	Thal minor	5–6%	0	—
$\alpha\alpha^{thal}$	Silent	1–2%	0	—
$\alpha^{thal_1}\alpha^{thal}$	Thal minor	5–6%	0*	—
$\alpha^{thal_1}\alpha^{thal_1}$	Hydrops fetalis	80–90%	±	A± Hb Portland <sup>27*</sup>
$\alpha^{thal_1}\alpha^{thal}$	Thal intermedia (Hb H disease)	25%	4–30	—
$\alpha\alpha^{CS}$	No abnormalities	0	0	<1% Hb <sup>CS</sup>
$\alpha^{thal_1}\alpha^{CS}$	Thal intermedia (Hb H disease)	—	13–19	Hb <sup>CS</sup> 2.5%

$\alpha$  = normal structural gene for  $\alpha$ -chain synthesis

$\alpha^{thal_1}$  = severe  $\alpha$ -thalassemia gene

$\alpha^{thal}$  = mild or 'silent'  $\alpha$ -thalassemia gene

$\alpha^{CS}$  = gene for Hb Constant Spring<sup>314</sup> (Hb Thai and Hb Athens may be identical)

\*See reference 307. The suggestion has been made that hemoglobin H disease may result from this genetic constitution.

completely reliable confirmatory tests in the absence of measurements of Hb Bart's levels at birth and/or measurements of globin-chain synthesis.

Normal newborn infants do not have levels of Hb Bart's in excess of 0.5%.<sup>56</sup> In Thailand, where an incidence of 20.4% Hb Bart's was found in newborn infants,<sup>317,318</sup> infants carrying 1 to 2% Bart's were designated as having  $\alpha$ -thalassemia<sub>2</sub> trait; those with 5 to 6%,  $\alpha$ -thalassemia<sub>1</sub> trait; and those with 25% or more as having Hb H disease, the latter disorder having been found on follow-up.<sup>341</sup> Infants with  $\alpha$ -thalassemia<sub>2</sub> trait had no hematologic abnormalities except significantly reduced MCH levels, whereas those with  $\alpha$ -thalassemia<sub>1</sub> trait had marked morphologic abnormalities of the red cells and a lower hemoglobin level than do normal infants. Hb Bart's concentrations in the cord blood of 17% of Yemenite Jewish infants and in that of 11% of Iraqi Jewish infants ranged from 1 to 6%.<sup>350</sup> In vitro measurements of  $\alpha$ -globin-chain synthesis in children who had

Hb Bart's levels of 5% at birth, the  $\alpha/\beta$  synthesis ratio was  $0.64 \pm 0.05$  (normal  $1.03 \pm 0.06$ ), as compared with a ratio of  $0.76 \pm 0.08$  in children with 2% Hb Bart's at birth.<sup>351</sup> The children were family members of patients with Hb H disease.

In Negroes in Baltimore<sup>283</sup> and in Africa,<sup>278,316</sup> measurements of Hb Bart's levels suggest that a form of  $\alpha$ -thalassemia which is associated with increased levels of Hb Bart's in the neonatal period may also exist in the Negro.<sup>316,335</sup> However, no typical case of Hb H disease or Hb Bart's hydrops fetalis has been described in the Negro.<sup>325</sup> Hb H disease, but no hydrops fetalis, has been reported in the Yemenite and Iraqi families.<sup>350</sup>

### Hb Bart's

Hb Bart's is a tetramer of the gamma chains of fetal hemoglobin ( $\gamma_4$ ).<sup>288</sup> A syndrome of hydrops fetalis was shown to be associated with levels of 80 to 90% Hb Bart's,<sup>299</sup> a hemoglobin which releases so

little oxygen to tissues that it is essentially useless as an oxygen-transport protein. This condition, the most devastating of all the thalassemias, is widely recognized in Southeast Asia.<sup>310,317,330,331,348</sup> The picture is that of a pale, bloated infant, either stillborn or dying within the first hour of life. Hepatomegaly is very marked, more so than splenomegaly.<sup>302</sup> There is marked anisopoikilocytosis and hypochromia, with numerous erythroblasts in the blood, variable reticulocytosis, and a hemoglobin level which ranges from 4 to 10 g/dl.

Starch gel electrophoresis of hemolysates at alkaline pH shows the hemoglobin to consist mainly of the moderately fast-moving Hb Bart's with smaller amounts of faster-moving Hb H. In the majority of cases, Hb A is absent. In addition there is an increased amount of a fetal hemoglobin of unique constitution ( $\zeta$ -chain), Hb Portland.<sup>372,332,345</sup>

The red cells of the parents of babies with the Hb Bart's hydrops fetalis syndrome show the morphologic changes of thalassemia. In addition, there are occasional cells containing inclusion bodies, findings consistent with the  $\alpha$ -thalassemia carrier state.<sup>292,319,341</sup> Hydrops fetalis thus appears to be the clinical consequence of severe, homozygous  $\alpha$ -thalassemia. This illness is more severe than homozygous  $\beta$ -thalassemia, presumably because, in the latter condition, the absence of  $\beta$ -chains can be partially compensated for by the presence of  $\gamma$ -chains, which produce hemoglobin F.

### Hemoglobin H Disease

Hemoglobin H disease is particularly prevalent in Southeast Asia,<sup>305,341</sup> the Middle East,<sup>307</sup> Greece,<sup>271,283</sup> and Cyprus.<sup>56</sup> It is found in Chinese<sup>304,323,336</sup> and has been reported sporadically in Italians (Sardinia),<sup>266,275</sup> Arabs,<sup>267</sup> Yemenite and Iraqi Jews,<sup>350</sup> and in North Europeans.<sup>285,349</sup> The clinical findings usually resemble those of thalassemia intermedia, but the manifestations vary in severity<sup>313</sup> from little disability to those of Cooley's anemia. Anemia is only mild at birth and increases during the first

year of life. Some degree of anemia is always present. The blood smear contains—in addition to microcytosis, hypochromia, and target cells—a number of tiny, misshapen red cells. Reticulocytosis (4 to 5%), polychromatophilia, stippling, and occasional Howell-Jolly bodies are found. Incubation of 3 or 4 drops of blood with 0.5 ml of 1% brilliant cresyl blue at room temperature results in the formation of intra-erythrocytic inclusion bodies (Fig. 26-11), formed by the precipitation of Hb H as the result of the redox action of the dye. Such precipitation has also been demonstrated in mature normoblasts in the bone marrow.<sup>279</sup>

Hb H, a tetramer of four normal  $\beta$ -chains ( $\beta_4$ ),<sup>200</sup> was the first reported of a series of "fast" hemoglobins<sup>323</sup> which share the electrophoretic characteristic of a faster anodal mobility at pH 8.6 than that of Hb A. It has a mobility similar to that of Hb I, from which it is distinguished by its anodal migration at pH 6.5. The total amount of Hb H in the red corpuscles of patients with Hb H disease has ranged from 4 to 30%.<sup>341</sup> A small quantity of Hb Bart's may be found<sup>286,321</sup> and Hb A<sub>2</sub> is always diminished (average 1.55%).<sup>341</sup> It was noted that, at birth, about 25% Hb Bart's was present, but this gradually decreased and was replaced by Hb H.<sup>341</sup> Although occasionally Hb Bart's levels may exceed those of Hb H.<sup>321</sup> In patients with Hb H disease in Thailand, on starch gel electrophoresis a cathodal band was described which migrated more slowly than Hb A<sub>2</sub>. This was designated Hb Thai<sup>337,342</sup> and may be the same as Hb Athens, or Hb Constant Spring (page 881), described in Greek<sup>328</sup> and Chinese<sup>277,308</sup> patients, respectively. Thus, from the standpoint of the clinical picture produced, the genes for these hemoglobins behave similarly to the "silent"  $\beta$ -thalassemia ( $\alpha$ thal) gene.

Hb H is relatively unstable and thermolabile.<sup>327</sup> Its oxygen affinity is ten times that of Hb A. There is no Bohr effect and its oxygen dissociation curve gives no evidence of heme-heme interaction.<sup>269</sup> These properties are disadvantageous to the anemic patient since less oxygen is given up at physiologic

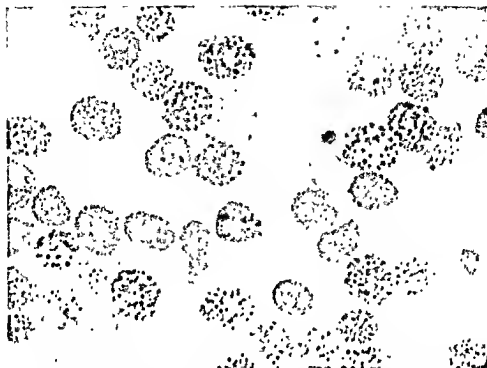


Fig. 26-11. Erythrocyte inclusion bodies in blood of patient with Hb H-thalassemia. The inclusion bodies appeared after incubation with brilliant cresyl blue for 20 minutes at 37°C and may be seen in nearly every erythrocyte in contradistinction to the reticulum of reticulocytes (dark black bodies in the photograph) which is present in only a few cells (X1000) (From Ottman et al.,<sup>275</sup> courtesy of the authors and Henry M. Stratton, Inc.)

tensions. The ease with which Hb H is oxidized, eg, by redox dyes, may be due to the presence of eight free thiols in its four  $\beta$ -chains.<sup>280,282</sup> One of the reasons for its tendency to precipitate may be the rapid rate at which it forms hemichromes.<sup>320</sup> In vitro studies of hemoglobin production have shown that  $\beta$ -chain synthesis exceeds that of  $\alpha$ -chains by a factor of 1.5/1 to 4/1.<sup>274,344</sup> Hb H red cells have increased membrane permeability,<sup>312</sup> the rate of loss of phospholipids on incubation is increased,<sup>327</sup> and their life span is greatly shortened.<sup>281,322</sup> The extreme degree of poikilocytosis seen in Hb H disease, as judged by electron microscopic studies,<sup>347</sup> is probably the result of removal of the inclusion bodies from the red cells as they pass through the reticuloendothelial system, as well as the splitting of the corpuscles into two or more fragments during their passage from splenic cords to sinuses.

As indicated previously, it is thought that Hb H disease results from the interaction of

at least two  $\alpha$ -thalassemia genes (one of these, presumably  $\alpha^{\text{thal}_2}$ , being so mild in heterozygotes as to be completely "silent" clinically<sup>293,296,303,311,313,339</sup>) or from double heterozygosity of Hb Constant Spring and an  $\alpha$ -thalassemia<sub>1</sub> gene<sup>342</sup> (Table 26-3). An alternative suggestion is that the disorder represents the homozygous state resulting from inheritance of the mild  $\alpha$ -thalassemia gene ( $\alpha^{\text{thal}_2}$ ) from each parent.<sup>307</sup> Studies in Thailand are in keeping with both these hypotheses.<sup>311,339</sup> With one exception<sup>294,295</sup> in which both parents were affected, only one parent of patients with Hb H disease has shown hematologic abnormalities in the blood smear and normal levels of Hb A<sub>2</sub>.

#### Hb H or Hb Bart's in Acquired Disorders

Hb H has been found in the red cells of some patients with "myeloproliferative" disorders,<sup>284</sup> especially erythroleukemia,<sup>324</sup> per-

haps as the result of imbalanced chain production consequent to a chromosomal aberration. Hb Bart's has been described in the  $D_1$  trisomy syndrome.<sup>287</sup>

#### $\alpha$ -Thalassemia in Association with $\alpha$ -Chain and $\beta$ -Chain Variants

The  $\alpha$ -chain variants reported in association with  $\alpha$ -thalassemia include Hb Constant Spring, mentioned above, Hb Q, and Hb I. Hb<sup>CS</sup> (*Constant Spring*)<sup>307a,308</sup> is an extremely long chain as it is made up of 31 additional residues attached to the C-terminal end of the 141 amino acids that normally make up the  $\alpha$ -chain. It is found in approximately 50% of persons with Hb H disease in Thailand and Malaysia and in 12% in Hong Kong, and behaves like an  $\alpha$ -thalassemia<sub>2</sub> gene.<sup>273</sup> Inherited together with an  $\alpha$ -thalassemia<sub>1</sub> gene, it produces the clinical picture of Hb H disease. A similar clinical picture was found in Hb Q  $\alpha$ -thalassemia and this, like Hb<sup>CS</sup>  $\alpha$ -thalassemia, was first reported in Chinese.<sup>301,336</sup> It has since been described in Thailand,<sup>276,306</sup> Iran,<sup>306</sup> and India.<sup>329</sup> In the Oriental Hb Q, the mutation (asp  $\rightarrow$  his) is at  $\alpha 74$  (EF3); in the Iranian, at  $\alpha 75$  (EF4)<sup>306</sup>; and in the Indian, at  $\alpha 64$ . These patients were found to synthesize no normal  $\alpha$ -chains and the abnormal  $\alpha^Q$ -chains combined with  $\beta$ - and  $\delta$ -chains to produce abnormal major ( $\alpha_2^Q\beta_2$ )- and minor ( $\alpha_2^Q\delta_2$ )-hemoglobin components. Hb I  $\alpha$ -thalassemia has been reported only in one family, all members of which were Negroes.<sup>268</sup>

The  $\beta$ -chain variants that have been found in association with  $\alpha$ -thalassemia are Hb S,<sup>335,352</sup> Hb C,<sup>191</sup> Hb E,<sup>333,334</sup> and Hb J<sup>Bangkok</sup>.<sup>341</sup> The combination of Hb S or of Hb C with  $\alpha$ -thalassemia appears to be associated with minimal anemia, but there are more marked morphologic changes of the red cells and lower levels of Hb S or Hb C, respectively, than would be expected in Hb S or Hb C heterozygotes.<sup>56</sup> The association of Hb E with  $\alpha$ -thalassemia, as might be expected from the frequency of these disorders in Southeast Asia, has resulted in many different combinations,<sup>340,341</sup> of which  $\alpha$ -thalassemia<sub>1</sub>/ $\alpha$ -thalassemia<sub>2</sub>/Hb E trait is the most

common.<sup>340</sup> Except for the presence of fewer inclusion bodies, the clinical picture is like that of Hb H disease.

$\alpha$ -Thalassemia also has been found in association with  $\beta$ -thalassemia,<sup>154,270,291</sup> ( $\delta\beta$ )-thalassemia,<sup>56</sup> and HPFH. Study of the clinical and hematologic manifestations of patients with different combinations of  $\alpha$ - and  $\beta$ -thalassemia permits the assumption that, in the absence of gross chain imbalance, marked hypochromic anemia is produced but the hemolytic element is much reduced, perhaps because membrane damage<sup>312</sup> is less.

#### Genetics of the $\alpha$ -Thalassemias

The genetics of the  $\alpha$ -thalassemias has been very ably discussed by Weatherall and Clegg<sup>56</sup> who point out that, in spite of the opportunities afforded by the study of the various combinations of genetic abnormalities, a number of questions remain to be answered before the genetic transmission of  $\alpha$ -thalassemia can be considered as being understood. It is not known whether there are one or two (or even more) structural loci on each chromosome controlling the production of the  $\alpha$ -chains of normal adult hemoglobin, or even whether the situation in this respect is the same in all populations. As stated previously, in the majority of patients with Hb H disease the condition appears to have resulted from the interaction of a recognizable and a "silent" gene.<sup>296,339</sup> The possible reduplication of  $\alpha$ -chain structural genes<sup>338</sup> was discussed in Chapter 24. It has been suggested that if there are two  $\alpha$ -chain loci on each chromosome, loss of two genes would produce the detectable carrier state; three genes, Hb H disease; and four, the Hb Bart's hydrops fetalis syndrome.<sup>294,297,338</sup> If unequal crossover occurs, one can conceive chromosomes that contain one or three genes, as distinguished from the normal two. Thus,  $\alpha$ -thalassemia might be visualized as resulting from the presence of only two or three  $\alpha$ -genes.<sup>298</sup> Since chromosomes are paired, five or six genes could result from chromosomes formed from such a crossover and this would produce an excess of  $\alpha$ -chains<sup>35</sup> (page 880).

### Treatment

What was said earlier in regard to the treatment of the  $\beta$ -thalassemia syndromes applies equally well to the treatment of  $\alpha$ -thalassemia. There have been reports of benefit from splenectomy in Hb H disease,<sup>283,341,349</sup> but results have not been uniformly good. Since Hb H is unstable and precipitates in the presence of oxidant drugs, the therapeutic use of such agents (Table 23-2 and Appendix C) should be avoided.<sup>322</sup>

*Genetic counseling* is especially important in Southeast Asia where  $\alpha$ -thalassemia represents a major public health problem. When a woman has lost a child with the Hb Bart's hydrops fetalis syndrome she probably has a one in four chance of producing similar infants through the same husband. A parent of a child with Hb H disease has a similar chance of passing the disease on to another offspring.

### Geographic and Racial Distribution of Thalassemia

As judged by such simple parameters as hematologic values, osmotic fragility studies, and Hb A<sub>2</sub> levels,  $\beta$ -thalassemia is found in a broad belt extending from the Mediterranean basin through the Middle East to the Far East<sup>365</sup> (see Fig. 24-7). It is very common in Italy and Greece. In Italy it is especially common in the delta of the Po River where, in some communities near Ferrara, its incidence was found to be as high as 20%.<sup>366</sup> It is also common in the far south and in adjoining Sicily (10% or more of Sicilians are carriers)<sup>36,358,379,380</sup> and in Sardinia (11 to 34% carriers).<sup>391</sup> In Greece<sup>326</sup> the distribution of the trait is irregular,<sup>367</sup> but frequency is high in the southern and central areas.<sup>353</sup> High incidence rates have been reported in Cyprus<sup>373</sup> and in Malta.<sup>381</sup> Thalassemia has been reported sporadically in northern and western parts of Africa.<sup>56,371</sup>

In the Middle East, many cases of  $\beta$ -thalassemia have been reported from Turkey,<sup>111</sup> Iran,<sup>368</sup> and Syria<sup>357,363</sup> and in Indian and Kurdish Jews.<sup>362,375</sup> It has been described in

Arabs, especially those in Saudi Arabia.<sup>56</sup> It is noted in Pakistan and in India,<sup>356,364</sup> especially in Bombay, Calcutta, and Madras. In the Far East, thalassemia is very common, both  $\beta$ - and  $\alpha$ -thalassemia having been reported.<sup>154,168,370</sup> As has already been discussed in some detail (page 879),  $\alpha$ -thalassemia is especially common in Southeast Asia. In the population of northern Thailand,<sup>370</sup> an incidence of 4.8 to 10% has been reported.<sup>156</sup> The total number of individuals affected with different combinations of thalassemia genes in Thailand has been estimated at 223,000.<sup>168</sup> The disease is also encountered frequently in both Chinese and Malays in the Malay Peninsula and in Indonesia, New Guinea, and western Melanesia, and has been described in India.<sup>359</sup> A number of cases of Hb H disease have been reported from the Middle East (page 879).

In the United States, thalassemia is noted particularly in persons of Italian<sup>371</sup> and Greek descent. In Negroes, although  $\beta$ - and  $\alpha$ -thalassemia<sup>383</sup> have been described, the incidence is low; the manifestations usually have been mild,<sup>356,358</sup> for reasons that are obscure.<sup>354,361</sup> Only once has the  $\beta$ <sup>thal</sup> gene been reported in the Negro.<sup>39</sup> Its presence in Jamaica may originate in the Oriental immigrants.<sup>258</sup> Thalassemia is rare in American Indians.<sup>378</sup>

Though rare, thalassemia has been recognized in North Europeans without Mediterranean or Oriental ancestry.<sup>56,358</sup>

The geographic distribution of thalassemia has aroused interesting speculation.<sup>378</sup> It has been attributed to migrations and commerce carrying the defect eastward from a large pool in the Mediterranean basin, or westward from Indochina; or it may have originated in Armenia and spread both to the east and west.<sup>353</sup> Why a potentially lethal gene like thalassemia has continued to be prevalent in certain areas is uncertain. It is less well established that thalassemia confers resistance to malaria than is the case for the sickle cell trait and G6PD deficiency (Chapter 23), but it is true that the high incidence areas for thalassemia are those in which malaria has been endemic.<sup>56,382</sup>



## Diagnosis and Differential Diagnosis of Thalassemia

Thalassemia is recognized by clinical examination, hematologic studies, hemoglobin electrophoresis, measurement of Hb A<sub>2</sub> and Hb F levels (Table 26-4), and family studies. The finding of microcytosis, hypochromia, basophilic stippling, and decreased osmotic fragility of the red corpuscles will alert the physician, whereas such findings as leukopenia and thrombocytopenia, low serum iron, and high iron-binding capacity direct attention to other disorders, such as Fanconi's anemia or iron-deficiency anemia. However, as mentioned earlier (page 871), if  $\beta$ -thalassemia is associated with iron deficiency, the Hb A<sub>2</sub> level falls, thereby leading to confusion. Hemoglobin electrophoresis will reveal the presence of Hb S, Hb C, Hb D, or Hb E, but does not exclude double heterozygosity for a hemoglobinopathy together with  $\beta$ -thalassemia. Marked hypochromia, microcytosis, and some stippling are found in sideroblastic anemias, but in those conditions

a double population of red corpuscles is likely to be noted (Chapter 18). A rapid screening test based on electronic size distribution curves has been reported to be very helpful in distinguishing thalassemia trait from both normal and sideropenic disorders.<sup>142</sup>

If splenectomy has already been carried out, inclusion bodies in the red cells will be found if one is dealing with unstable hemoglobin (Chapter 24) and if the associated hemoglobin variant has a neutral amino acid substitution, the heat precipitability test will be needed to distinguish an unstable hemoglobin from thalassemia.

Hb F levels may be markedly increased in acquired conditions, such as the di Guglielmo syndrome (Chapter 18) and juvenile chronic myelocytic leukemia.<sup>56</sup> In sideroblastic anemias, Hb F may be somewhat increased and Hb A<sub>2</sub> usually is low. Hb F also may be slightly increased in pernicious anemia, myelofibrosis, aplastic anemia, and even in paroxysmal nocturnal hemoglobinuria.<sup>56</sup> Hb A<sub>2</sub> levels may be increased in megaloblastic anemias.<sup>56</sup> Careful clinical examina-

Table 26-4. Hemoglobin Constitution in Some of the Thalassemias

	RBC Morphology	Hb	Hb A <sub>2</sub>	Hb F	Abnormal Hb
<b>Homozygotes</b>					
$\alpha$ -thalassemia	Thalassemic	—	—	Variable	Hb Bart's
$\beta$ -thalassemia	Thalassemic	—, 0	+ N, —	++	$\alpha$ -chains
( $\delta\beta$ ) thalassemia	Thalassemic	0	0	++++	0
$\delta$ -thalassemia	Normal	N	0	N	0
Hb Lepore	Thalassemic	0	0	++	Hb Lepore
HPFH	Normal	0	0	100%	0
<b>Heterozygotes</b>					
$\alpha$ -thalassemia	Thalassemic	+	N	N	Hb Bart's $\pm$ Hb H $\pm$
$\beta$ -thalassemia	Thalassemic	N —	++	N (+)	0
( $\delta\beta$ )-thalassemia	Thalassemic	N, —	N	++	0
$\delta$ thalassemia	Normal	N	—	N	0
Hb Lepore	Thalassemic	N, —	—	+	Hb Lepore
HPFH	Normal	—	—	++ (Homogeneous distribution)	0
<b>Double heterozygotes</b>					
Hb S $\beta$ thalassemia	Thalassemic; sickling	—	+	+	Hb S
Hb E $\beta$ -thalassemia	Thalassemic	0	0?	+	Hb E
Hb E $\alpha$ thalassemia	Thalassemic; Hb H	+	?	+	Hb E
					Hb Bart's in some

tion will almost always make differentiation possible.

To differentiate the various forms of thalassemia, family studies and relatively sophisticated laboratory tests are needed. The absence of any anemia and the normal appearance of the red cells in hereditary persistence of fetal hemoglobin (HPFH) distinguish this condition from homozygous ( $\delta\beta$ )-thalassemia. Its differentiation from  $\beta$ -thalassemia minor was discussed earlier (page 876). The combination of  $\alpha$ -thalassemia with HPFH may produce a picture similar to that of heterozygous ( $\delta\beta$ )-thalassemia, but Hb H inclusions or the presence of small amounts of Hb Bart's on starch gel electrophoresis point to the coexistence of  $\alpha$ -thalassemia.

Detection of the  $\beta$ -thalassemia trait at birth was accomplished by measuring the incorporation of  $^{14}\text{C}$ -leucine into the  $\alpha$ -,  $\beta$ -, and  $\gamma$ -chains. In an affected infant, the presence of one  $\beta$ -thalassemia gene was revealed on the first day of life by the lower specific activity of the  $\beta$ -chain.<sup>122</sup> In the red cell precursors of newborn infants with  $\beta$ -thalassemia, inclusion bodies have been observed.<sup>61</sup>

As discussed earlier (page 876) the  $\alpha$ -thalassemia traits are very difficult to recognize, since a defect in  $\alpha$ -chain synthesis affects equally the synthesis of hemoglobins A, A<sub>2</sub>, and F. The measurement of Hb Bart's in the neonatal period is very helpful, but in adult life the most that can be expected is slight hypochromia and minor changes in the red cell indices. Hb H disease (page 879) is usually recognized by the association of a thalassemia-like picture with intracellular inclusions precipitated by brilliant cresyl blue (Fig. 26-11). A history of repeated hydropic stillbirths in an Oriental woman strongly suggests that Hb Bart's is the cause. The hemoglobin pattern of the stillborn or examination of blood obtained by amniocentesis of a suspected pregnant woman will reveal the abnormal hemoglobin if this, rather than neonatal hemolytic disease due to Rhesus or ABO incompatibility, is the cause.

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## *Immunohemolytic Anemias*

Incompatible Transfusion Reactions  
Hemolytic Disease of the Newborn  
Hemolytic Disease of the Newborn Due to  
ABO Incompatibility  
Immunochemolytic Anemias Due to Warm  
Reactive Antibodies  
Drug-Induced Immunochemolytic Anemias  
Immunochemolytic Anemias Due to Cold  
Reactive Antibodies

### **Incompatible Transfusion Reactions**

The clinical aspects of transfusion reactions were discussed in Chapter 11. The mechanisms of hemolysis due to ABO and Rh antibodies will be considered here because they serve as prototypes of the reactions occurring in other acquired immunochemolytic anemias.

#### **Red Cell Destruction by ABO Antibodies**

Hemolytic reactions due to ABO incompatibility are brought about by isoantibodies with exquisite specificity for ABO blood group substances. These antibodies develop early in life in response to repeated exposure to ABO-like substances found in food, bacteria and other exogenous materials and are always found in the serum when the corresponding antigen is lacking on the red cells

(Table 11-1). Thus prior transfusion is not necessary for sensitization to occur. Although ABO antibodies may belong to any of the major immunoglobulin classes, group A and group B subjects are known to have predominantly IgM anti-B and anti-A antibodies, respectively, whereas group O subjects commonly develop IgG antibodies as well.<sup>107</sup> When antibodies are present in low concentrations they appear to have only agglutinating properties *in vitro*, hemolytic activity being undetectable with present techniques. When present in higher concentrations, both the IgM and IgG antibodies can be shown to have complement-dependent lytic activity *in vitro*<sup>107</sup>; in the absence of complement, only the agglutinating properties are demonstrable. Because these antibodies are capable of agglutinating red cells suspended in saline solution, they are termed "complete antibodies."

Jandl<sup>9</sup> and others<sup>8,13,15</sup> conducted illuminating studies on the fate of <sup>51</sup>Cr-labeled red cells infused into ABO incompatible recipients. When small volumes of such cells are infused into individuals with normal levels of isoagglutinins but no demonstrable isohemolysin activity, the red cells are quickly removed from the circulation, half the labeled erythrocytes being cleared in just under two minutes. Moderate hemoglobinemias develop rapidly and surface counting shows marked and rapid accumulation of radioactivity over the liver.

In subjects with demonstrable agglutinin and low levels of hemolysin activity the pattern is similar except that high levels of  $^{51}\text{Cr}$  and hemoglobin appear in the plasma immediately after the injection of cells and decline during the next several hours. In subjects with high levels of hemolysins, hemoglobinaemia appears abruptly and is pronounced, reflecting the virtually instantaneous destruction of incompatible cells by complement-dependent mechanisms. At least 80% of the hemoglobin contained in the injected cells can be found in the plasma, peak levels being reached in less than 60 seconds. Under these circumstances, accumulation of radioactivity over the liver is considerably slower and less marked than in the presence of isoagglutinins only. As described in Chapter 5, the free hemoglobin or heme is removed by renal excretion or by complexing to haptoglobin, hemopexin, or albumin, with subsequent clearance by the reticuloendothelial system (RES); the red cell membrane most likely is also removed by the RES, predominantly in the liver and the spleen.

The observations just described were made when relatively small volumes of cells were being destroyed under conditions of excess antibody, complement, and other serum factors. Under appropriate circumstances the destruction of larger numbers of cells has been shown to proceed more slowly because the number of erythrocytes introduced into the circulation may significantly decrease the concentration of available antibody.<sup>107</sup> This applies especially to ABO-incompatible transfusions since the number of A and B antigen sites per red cell is considerable, being at least 100 times greater than, for instance, the number of D-antigen sites.<sup>213,254-251</sup> When the serum contains potent antibodies, however, even large ABO-incompatible transfusions may not remove all anti-red cell activity from the circulation.

#### Red Cell Destruction by Rh Antibodies

Antibodies to Rh antigens differ significantly from anti-A or anti-B antibodies.

1. Anti-Rh antibodies do not occur "naturally" but are synthesized in response to stimulation by foreign red cells bearing the appropriate antigenic determinants.

2. Anti-Rh antibodies are predominantly IgG globulins,<sup>107</sup> although, early in the immune response, gamma M antibodies may also be prominent. Only occasionally are IgA Rh antibodies encountered.<sup>107</sup>

3. Antibodies to Rh antigen usually do not fix complement and are rarely lytic in vitro<sup>25,26</sup> even though Rh antibodies were shown to belong predominantly to the IgG1 and IgG3 subclasses and are therefore structurally equipped for complement fixation<sup>269,271</sup> (Chapter 7). It is possible that the lack of complement fixation by the red cell-bound anti-Rh antibodies is related to the relative sparsity of specific Rh antigenic sites on the cell surface,<sup>281</sup> since two adjacent IgG molecules are required for activation of complement to occur.<sup>4</sup> In keeping with this interpretation, complement activation can be demonstrated if red cells are treated with a combination of specific antisera, for instance anti-hr' (c), anti-Rh<sub>0</sub> (D), anti-rh" (E)<sup>22</sup>

4. With the exception of the early IgM antibodies, anti-Rh antibodies do not lead to agglutination of red cells in saline solution and are therefore considered to be "incomplete" antibodies. This phenomenon is not fully understood but may be related to the molecular length of the IgG antibody,<sup>20</sup> the relative sparsity of antigenic sites per red cell,<sup>281</sup> or other repulsive forces that prevent the erythrocytes from approaching close enough to allow the antibody to bridge the gap.<sup>19</sup> Such repulsive forces include the like negative charge on the surface of all erythrocytes, and the cloud of cations attracted by the surface negative charge; the net potential of these two layers is normally negative, is directed toward the outer aspect of the cloud, and determines the net repelling force between two cells. It is referred to as the zeta potential and equals 15 millivolts in isotonic solutions; the maximal zeta potential at which human red cells can be agglutinated by "complete" (IgM) agglutinins is 18 to 20 millivolts, while for incomplete (IgG) an-

tibodies it is only 8 to 9 millivolts.<sup>20</sup> Albumin and other colloids such as fibrinogen or polyvinyl pyrrolidone reduce the zeta potential by their higher dielectric constants that drain away the net negative charge and thereby enhance the agglutinating potential of incomplete antibodies.

### Coombs' Antiglobulin Tests

The presence of anti-Rh antibodies on the surface of red cells is most readily demonstrated by means of the *direct Coombs' test* (direct antiglobulin test) (Fig. 27-1). The reagents for this test are prepared by immunizing rabbits with whole human serum, gamma globulin, or complement. The result-

ant antisera may be appropriately absorbed to yield reagents with specificity for a given gamma globulin class or complement. When cells sensitized with incomplete antibodies or complement components are suspended in the appropriate (Coombs') antiserum, agglutination of the cells results. Nonsensitized cells so treated do not agglutinate, although rare exceptions have been noted.<sup>23</sup>

The technique of the direct test has been modified to detect anti-red cell antibodies present in the serum; this test is known as the *indirect Coombs' test* (indirect antiglobulin test). In this procedure, various types of normal red cells are incubated in the patient's serum under appropriate conditions. After being washed free of nonspecifically adherent

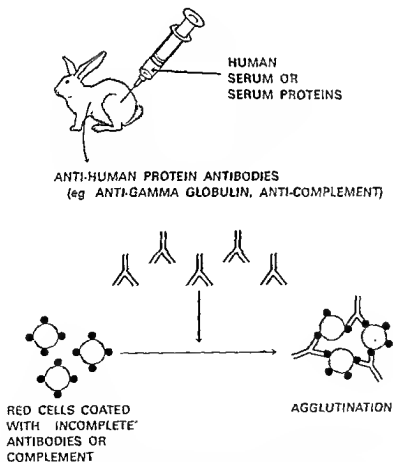


Fig 27-1. Direct Coombs' test Rabbits or goats are immunized with human serum or serum components. The resulting sera containing anti-gamma globulin or anti-complement antibodies are then added to test samples of red cells. If human gamma globulins and/or complement components are bound to the cell surface, agglutination occurs.

gamma globulin or complement they are tested by Coombs' serum as in the direct procedure. Agglutination indicates the sensitization of normal cells by gamma globulin, or complement components, depending on the type of Coombs' antiserum used. As discussed elsewhere (page 472) the indirect antiglobulin test can be made more sensitive by treating normal test cells with proteolytic enzymes. This manipulation may even lead to agglutination of cells by incomplete antibodies in the absence of antiglobulin sera.

### Site of Destruction

When small volumes of  $^{51}\text{Cr}$ -tagged  $\text{Rh}_0(\text{D})$  red cells are injected into individuals with relatively high titers of incomplete anti- $\text{Rh}_0$  antibodies, the labeled erythrocytes are quickly removed from the circulation and there is a concomitant increase of radioactivity over the spleen and to a much smaller degree over the liver.<sup>3,6,9</sup> The half-life of these cells in the circulation is only a few minutes and very little hemoglobin or nonerythrocyte  $^{51}\text{Cr}$  appears in the plasma, attesting to the predominantly extravascular destruction of red cells coated by incomplete antibodies. Even when larger volumes of Rh-incompatible red cells are given, the circulating hemoglobin does not reach levels higher than those that would be derived from 12% of the total number of cells infused; in addition, the peak of extracellular hemoglobin is reached an hour after transfusion, suggesting that intravascular spillage occurs only when the mechanisms for removal of extravascularly released heme proteins have been saturated.<sup>9</sup> The mechanism of hemoglobin release under these circumstances is obscure.

Extravascular splenic destruction of anti-Rh antibody-coated red cells occurs over a wide range of antibody concentrations.<sup>16</sup> The speed of removal from the circulation appears to depend on the amount of antibody coating the red cells: antibody concentrations of the order of 25/ $\mu\text{g}/\text{ml}$  of red cells (corresponding to a titer of  $1/64$  or more) are required to bring about their clearance with a single passage through the spleen, the clear-

ance half-time being about 20 minutes. Antibody concentrations of less than 5  $\mu\text{g}/\text{ml}$  of red cells and corresponding to titers of  $1/8$  or less may bring about the removal of red cells in a half-time of 60 to 100 minutes.<sup>6,16</sup> The liver clears red cells coated with "incomplete antibodies" much less efficiently than does the spleen.<sup>16</sup> Even when erythrocytes are sensitized with as much as 40  $\mu\text{g}$  of antibody per milliliter of red cells, only about one third are cleared by a single passage through the liver. Nevertheless, the liver plays a *clinically* significant role in the destruction of red cells, especially of those coated by high concentrations of antibody.<sup>213a</sup>

The way in which the RES destroys anti-Rh antibody-tagged erythrocytes is not fully understood. LoBuglio et al.<sup>11</sup> and others<sup>2</sup> demonstrated, *in vitro*, the binding of antibody-coated red cells to monocytes and macrophages, accompanied by rapid phagocytosis and red cell fragmentation. The receptor site on the macrophage has specificity for the Fc portion of the IgG molecule and seems to be distinctly different from the site that binds IgM-complement-coated red cells. A further mechanism of splenic red cell destruction is suggested by the observation that agglutinates consisting of red cells coated by incomplete antibodies are numerous in splenic blood and may indeed escape into the peripheral circulation.<sup>245</sup> It is likely that the high VPRC of splenic blood and the higher protein concentration of the intrasplenic plasma<sup>24</sup> create conditions that induce agglutination of sensitized cells (see above) and their subsequent sequestration. Because of the metabolically unfavorable environment of the spleen<sup>27</sup> and the partial phagocytosis by macrophages,<sup>5</sup> spherocytes may result; because of their structural rigidity the spherocytes are then destroyed by mechanisms described elsewhere (see Chapter 21). Once the cells have been sequestered, destruction appears to be complete within minutes.

The most important features of red cell destruction by "complete" (IgM) and "incomplete" (IgG) antibodies are summarized in Table 27-1.

The clinical manifestations, diagnosis, and

**Table 27-1. Red Cell Destruction by "Complete" (IgM) and "Incomplete" (IgG) Antibodies**

Antibody	Predominant Site of RBC Destruction			Complement Dependency	Hemoglobinemia	Bilirubinemia	Specificity of Coombs' Test
	Intravascular	Extravascular					
		Liver	Spleen				
Low titer anti-A or anti-B (IgM)	±	+	—	±	±	+	Anti-C'
High titer anti-A or anti-B (IgM)	+	—	—	+	+	+	Anti-C'
Low titer anti-D (IgG)	—	—	+	—	—	+	Anti-IgG
High titer anti-D (IgG)	—	±	+	—	±	+	Anti-IgG

treatment of transfusion reactions are discussed in Chapter 11.

## Hemolytic Disease of the Newborn (HDN)

In an earlier chapter (page 458) the discovery of the Rh blood group system<sup>95</sup> and the suggested role of anti-Rh antibody in the pathogenesis of HDN<sup>97</sup> were described. Since that discovery it has been learned that HDN may be produced in a number of other ways as well (Table 27-2). This discussion, however, will deal almost exclusively with hemolysis caused by maternal antibodies against fetal cells.

### Etiology and Pathogenesis

When fetal red cells cross the placenta, they may stimulate the production of maternal antibodies against those fetal antigens that are not inherited from the mother and are therefore regarded as foreign. Some of these antibodies then cross into the fetal circulation and cause the destruction of fetal red cells. Fetal-maternal ABO incompatibility is responsible for two thirds of all cases of HDN,

but Rh incompatibility, which accounts for most of the remainder, is clinically the more important because it causes disease of far greater severity.<sup>36</sup> A few cases, perhaps 2% of the total, involve minor antigens such as hr'(c), rh''(E), rh''<sup>W2</sup>(E<sup>W</sup>), and Kell.<sup>122</sup> Most of the following discussion will deal with hemolytic disease due to Rh incompatibility. That due to ABO incompatibility will be discussed separately (page 908).

### Passage of Fetal Cells into the Maternal Circulation

Direct proof that fetal erythrocytes enter the maternal circulation was obtained in 1954 when Chown demonstrated the transfer of large amounts of blood (160 ml) by use of the differential agglutination technique.<sup>54</sup> Subsequently a more sensitive method for the detection of fetal erythrocytes was developed by Kleihauer, Braum, and Betke<sup>91</sup>; this method depends on the demonstration of cells containing hemoglobin F by the acid elution technique. The test is a sensitive one, making possible the detection of as little as 0.05 to 0.1 ml of fetal blood in the maternal circulation. By its use, small numbers of fetal cells have been demonstrated in the maternal

**Table 27-2. Causes of Unconjugated Neonatal Hyperbilirubinemia**


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I	Physiologic jaundice
II	Hemolytic diseases
A	Hemolytic disease of the newborn due to iso-antibodies
1	Rh, ABO and minor blood group incompatibilities
2	Maternal autoimmune hemolytic anemia
B	Hemolytic disease of the newborn due to inherited intracorpuscular defects
1	Hereditary spherocytosis
2	Enzyme deficiencies
	G-6PD
	Pyruvate kinase, etc
C	Drugs and chemicals
1	Vitamin K <sub>2</sub>
2	Naphthalene
3	Novobiocin
III	Neonatal sepsis
A	Bacterial sepsis
B	Viral infections
1	Cytomegalic inclusion disease
2	Congenital rubella
3	Disseminated herpes simplex
C	Congenital toxoplasmosis
D	Congenital syphilis
IV	Resorption of large hematomas
V	Metabolic disorders
A	Galactosamia
B	Crigler-Najjar syndrome
C	Breast milk jaundice
D	Transient familial neonatal hyperbilirubinemia
E	Maternal diabetes

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circulation as early as the third month of pregnancy.<sup>59, 167</sup> However, the chances of finding fetal cells in mothers' blood appear to increase as pregnancy progresses; an incidence of 28.9% in the third trimester was reported.<sup>59</sup> Others noted an overall incidence of 15.8% throughout pregnancy<sup>167</sup> and observed that the frequency with which fetal cells were found increased with the number of examinations made, reaching 43.6% in those examined five times.<sup>167</sup> Thus the transplacental passage of small amounts of fetal blood (less than 0.1 ml) is a very common event during normal pregnancy. Larger volumes of fetal blood (greater than 0.1 ml) may enter the maternal circulation at the time of delivery<sup>161, 165</sup> and as the result of various obstetric manipulations such as cesarean sec-

tion,<sup>156, 167</sup> manual removal of the placenta,<sup>127, 156, 167</sup> and perhaps the use of general anesthesia, forceps, and oxytocics.<sup>128</sup> Amniocentesis with placental injury seems to result in the transfer of unusually large amounts of blood into the maternal circulation<sup>166</sup>; this accident was observed in 4 of 13 women studied by Zipursky,<sup>168</sup> resulting in estimated losses of 10 to 50 ml of fetal blood.

### *Factors Influencing the Production of Anti-Rh Antibodies*

**SIZE OF THE TRANSPLENTAL HEMORRHAGE.** The amount of transplacental bleeding is of major importance.<sup>103, 160, 165</sup> Theoretically, sensitization of the Rh-negative mother exposed to Rh-positive cells could occur either as a result of small "normal" hemorrhages occurring during the course of pregnancy, or as a result of major bleeding occurring under the special circumstances outlined above.<sup>169</sup> Current data indicate that small transplacental hemorrhages constitute the most common cause of Rh immunization.<sup>163</sup>

One study showed that 19 of 472 Rh-negative women at risk developed antibodies within six months of delivery.<sup>163</sup> The blood of 14 of these 19 (73%) contained less than 0.1 ml of fetal cells immediately postpartum; the remaining 5 had 0.1 ml or more. Nevertheless the risk of developing antibodies is five times greater with the larger (>0.1 ml) than with the smaller (<0.1 ml) amount of bleeding—5 of 32 (15.6%) versus 14 of 440 (3%), respectively. When the transplacental hemorrhage is in excess of 3 ml, the risk of sensitization may be as high as 50%.<sup>103</sup> In addition, the same quantity of cells seems to be more immunogenic if given as a single dose, rather than a series of small doses distributed over a prolonged period, which seems to be the usual occurrence in a normal pregnancy. Why so many Rh-negative women exposed to Rh-positive cells do not develop antibodies, especially during their first pregnancy, is poorly understood. In addition to the size of the red cell inoculum and its time-dose relationship (discussed above),

other factors may be involved. Thus, a small number of Rh-negative individuals apparently do not produce detectable antibodies, even in response to relatively large Rh-positive blood transfusions. It is therefore possible that some individuals may be incapable of responding to certain Rh antigens. If true, this situation may be analogous to the genetically determined immune defects described in many animal systems.<sup>260</sup> The degree to which such failure to produce antibodies contributes to the relatively low incidence of Rh sensitization is not known.

It is also possible that sensitization may have taken place but may be undetectable by present diagnostic methods, which depend on the demonstration of fairly large quantities of antibody. This phenomenon has been termed "sensibilization"<sup>1169</sup> and, if it is valid, may explain the curious observation that antibodies are not usually seen during the first ("immunizing") pregnancy but are found during subsequent pregnancies.

**EFFECT OF ABO INCOMPATIBILITY ON RH SENSITIZATION.** In 1943, Levine noted a deficiency of ABO-incompatible matings among the parents of infants with HDN and suggested that this incompatibility afforded protection against hemolysis by anti-Rh antibodies.<sup>141</sup> Presumably, fetal cells would be destroyed by the "natural" anti-A or anti-B antibodies in the maternal circulation before sensitization to Rh could take place. It has been found, however, that this protection is not absolute, as Rh sensitization has been described across the ABO-incompatibility barrier,<sup>65</sup> although usually with low titers of anti-Rh antibody<sup>114</sup> and less severely affected infants.<sup>145</sup> Protection seems to be more complete from anti-A (90%) than from anti-B (55%),<sup>114</sup> which is in keeping with the observations that fetal B cells may survive for prolonged periods in the circulation of A<sub>1</sub> or O mothers even in the presence of anti-B antibody.<sup>58</sup> Antigenic heterogeneity of the B system similar to that of the A system (Chapter 11) has been postulated to explain these findings.

An additional factor limiting the incidence

of maternal sensitization is *Rh heterozygosity of the father*. The approximate chances of eventual sensitization to Rh by pregnancy, both with or without ABO incompatibility, are detailed in Table 27-3.

### Clinical Manifestations

The most important clinical manifestations of HDN are anemia, jaundice, hepatosplenomegaly, and, in untreated infants, bilirubin encephalopathy (kernicterus). The consequences of the disease range from death to a barely perceptible hemolytic process. The various clinical manifestations are all interdependent in terms of their genesis and the severity of their clinical expression; thus a severe degree of anemia is usually associated with equally severe hyperbilirubinemia and a high risk of central nervous system complications, whereas mild anemia will likely be accompanied by relatively less jaundice and fewer other complications.

### Anemia

Most infants suffering from HDN have good color at birth and the cord hemoglobin may only be at the lower limits of the normal range. However, the hemoglobin of most affected infants begins to fall during the first 24 hours of life, and pallor may be obvious by the second day. The degree of anemia that

**Table 27-3. Approximate Chances of Sensitization to Rh by Pregnancy under Various Conditions of Zygosity and ABO Compatibility of the Husband**

Husband's Zygosity for Rh	ABO Compatibility of Husband		ABO Type of Husband Unknown
	Incompatible (%)	Compatible (%)	
Heterozygous	1	3	2
Homozygous	4-5	11	9
Zygosity unknown	2-3	7-8	5

From Allen and Diamond <sup>34</sup> courtesy of the authors and Little, Brown & Company

develops reflects the balance between the rate of red cell destruction and production, and, in most affected infants, production is incapable of keeping up with destruction. When this imbalance becomes extreme, pallor may be very marked and may be accompanied by signs of congestive heart failure, including tachycardia, weak heart sounds, elevation of central venous pressure, massive generalized edema, ascites, pleural effusions, and marked hepatosplenomegaly. This clinical syndrome is referred to as *hydrops fetalis* and survival beyond a few hours is unusual. Indeed, most infants so afflicted die in utero, and this still constitutes the most important cause of death associated with hemolytic disease of the newborn.<sup>321</sup>

Occasionally the onset of anemia is delayed beyond the immediate neonatal period.<sup>122</sup> Under these circumstances it may be due to a slow but relentlessly progressive hemolytic process that does not require early exchange transfusions but may nevertheless lead to severe and sometimes fatal anemia after the second or third week of life. More commonly, however, despite exchange transfusions (see below), anemia of delayed onset occurs with a gradual decrease of hemoglobin levels to 5 or 6 g/dl at four to six weeks of life.<sup>122</sup> This anemia is not well understood but may be due to persistence of anti-Rh antibodies or decreased red cell production. The latter possibility is suggested by the low reticulocyte counts characteristically noted. The anemia lessens spontaneously at about eight weeks and seldom requires transfusions. Iron and vitamins, such as folic acid and vitamin B<sub>12</sub>, are of no therapeutic value.

### Jaundice

Since bilirubin is readily transferred across the placenta,<sup>96, 132</sup> most infants with HDN are not jaundiced at birth. Icterus usually develops during the first 24 hours of life; it is undoubtedly related to the loss of the placental excretory mechanism and the low level of glucuronyl transferase activity in the neonatal liver<sup>48</sup> which leads to an accumulation

of unconjugated bilirubin in the patient's blood and tissues. These functional defects may be particularly marked in premature infants<sup>45</sup> and may explain why even minimal degrees of hemolysis may lead to marked hyperbilirubinemia in these patients. In untreated infants with relatively mild disease the bilirubin level peaks by the fourth or fifth day and then declines slowly.

Since an early diagnosis of jaundice is of great clinical importance, extreme care should be taken in its detection. The baby should be examined under daylight or white fluorescent light and the skin should be blanched with a glass slide to compress the capillaries, whose pink hue obscures the yellow tinge of the jaundiced skin.

When jaundice occurs in the first 24 hours of life, it is usually due to hemolytic disease. That due to Rh antibodies must be differentiated from jaundice due to other types of antibodies,<sup>33</sup> as well as other causes of accelerated red cell destruction; these in turn must be differentiated from nonhemolytic hyperbilirubinemia occurring in the neonatal period (Table 27-2).

### Bilirubin Encephalopathy (Kernicterus)

An important complication of severe indirect hyperbilirubinemia in the neonatal period is the development of bilirubin encephalopathy.<sup>93, 112</sup> The term "kernicterus" more properly describes the macroscopic yellow staining of certain cerebral nuclei. Bilirubin encephalopathy may, of course, accompany indirect hyperbilirubinemia resulting from any cause. Initially the baby becomes lethargic and hypotonic and loses the sucking reflex, as a result of which it feeds poorly. In addition it may also roll its eyes and develop an unpleasant high-pitched cry. Later, opisthotonos and generalized spasticity are seen and at this stage most of these babies (70%) also develop irregular respirations that may be accompanied by pulmonary hemorrhage with frothy pink sputum. This may lead to death, usually within 12 hours of the onset



of pulmonary complications. Babies without pulmonary disturbances often survive for months or years but may suffer from the post-kernicterus syndrome, which includes high-frequency nerve deafness, athetoid cerebral palsy, and dental enamel dysplasia.<sup>122</sup> Surviving infants may lose some of their spasticity during the second week of life and this may lead physicians to conclude erroneously that brain damage has not occurred.<sup>107,122,123</sup> The later the onset of cerebral damage, however, the more likely is the child to live.<sup>76</sup>

There is a close relationship between the severity of the jaundice and the development of kernicterus. In one study, 8 of 11 infants with maximum bilirubin levels of 30 to 40 mg/dl developed kernicterus, whereas the corresponding incidence at bilirubin levels of 25 to 29, 19 to 24, and 10 to 18 mg/dl was four out of 12, one out of 13, and none out of 24 respectively.<sup>107</sup>

### *Hepatosplenomegaly*

Hepatosplenomegaly almost invariably accompanies hemolytic disease of the newborn and its degree reflects the severity of the underlying disease. The greatest degree of hepatosplenomegaly with accompanying ascites is seen in association with hydrops fetalis (see above). Secondarily induced hepatic damage may in turn contribute to the poor handling of bilirubin by the liver, thus introducing a vicious cycle into the pathogenetic mechanisms of this disease. Fortunately, infants who survive severe HDN seem to have no clinical or laboratory evidence of liver disease in later years.<sup>40</sup>

### *Purpura*

Purpura due to thrombocytopenia is seen in severely affected infants and is usually a bad prognostic sign. It is not clear whether the purpura is due to concomitant platelet destruction by isoantibodies or to other mechanisms, or whether it is due to decreased platelet production.

## **Laboratory Findings**

### *Blood*

Anemia, reticulocytosis, and nucleated red cells constitute the main findings in the peripheral blood. Initial determinations are usually obtained from samples of venous blood from the cord, which accurately reflect the blood findings in the infant. Only about half of all infants with hemolytic disease of the newborn have hemoglobin levels below 14 g/dl at birth,<sup>107</sup> the lower limit of normal for newborn infants<sup>121,122</sup>; indeed 11% of infants with hemolytic disease of the newborn have cord hemoglobin levels greater than 17.5 g/dl. Nevertheless, there is a rough correlation between the initial hemoglobin levels and the severity of the disease process.<sup>107</sup> In hydrops fetalis the hemoglobin concentration may be as low as 3 g/dl. Untreated patients, even those with relatively high levels of hemoglobin initially, may experience a rapid fall of the hemoglobin level after birth, often at rates of 3 g/dl or more per day.<sup>324</sup> Because of early treatment, these dramatic changes are rarely seen at the present time.

The erythrocytes are macrocytic and well filled with hemoglobin. Measurements of the red cell diameter show a biphasic curve with a macrocytic and a normocytic peak or two macrocytic peaks.<sup>150</sup> There is little poikilocytosis. Spherocytosis is usually not found in infants with HDN due to anti-Rh antibodies, although it is a characteristic feature in those with HDN due to ABO incompatibility (page 909). Polychromatophilia usually is marked. The reticulocyte count may be as high as 60%. This count (expressed as a percentage) is thought to be a poor guide to the severity of the disease or the need for transfusion therapy,<sup>107,324</sup> but studies that relate various parameters of the disease to the more meaningful absolute reticulocyte count have not been reported in the literature.

One of the most striking findings in the peripheral blood of infants suffering from HDN is the great increase in nucleated red cell precursors ("*erythroblasts fetalis*"). There may be from 10 to 100 × 10<sup>9</sup>/l nu-

cleated red cells<sup>62</sup> as compared with  $0.2$  to  $2 \times 10^9$  in normal premature or full-term infants. The nucleated cells represent all stages of maturation, although mature forms are by far the most common. They are often very large but are not megaloblastic as has been claimed by some writers.<sup>130</sup> Their size is probably a reflection of the rate of erythropoiesis.

A pronounced *leukocytosis* frequently accompanies hemolytic disease of the newborn, and counts in excess of  $30 \times 10^9/l$  have been reported.<sup>321</sup> It must be remembered, however, that the white cell count at birth may normally range between  $15$  and  $20 \times 10^9/l$ , with slightly lower counts in premature infants.<sup>121</sup> Leukocytosis is most marked in those infants who are most severely affected and are very anemic. The leukocytosis is predominantly due to an increase in neutrophils, and a considerable left shift is frequently noted.

*Platelet* counts may show normal values, but, in infants with severe disease, profound thrombocytopenia may be present.

### Serum Bilirubin Levels—

Since bilirubin is readily transferred across the placenta<sup>96,132</sup> the bilirubin levels of cord plasma do not give a complete picture of the severity of the hemolytic process. Nevertheless there is some correlation between cord bilirubin concentration and the severity of the disease. This observation may be particularly valuable when the cord hemoglobin concentration, which in most cases is a more reliable indicator of severity, is within normal limits.<sup>107</sup> Cord bilirubin values above  $4$  mg/dl are unusual and when present suggest very severe disease.<sup>122</sup>

After birth the bilirubin level reflects both the severity of the hemolytic process and the immaturity of the liver enzyme system responsible for conjugation and excretion of bilirubin. Peak bilirubin levels are usually reached by the third or fourth day of life and may attain values of  $40$  to  $50$  mg/dl.

Under most circumstances the hyperbilirubinemia is predominantly due to unconju-

gated indirect-reacting pigment. Occasionally, however, prolonged jaundice occurs in association with direct-reacting hyperbilirubinemia; this phenomenon has been referred to as the "*inspissated bile syndrome*." It may accompany a variety of clinical conditions including HDN. In one large series, the inspissated bile syndrome was present in 15% of all patients with hyperbilirubinemia.<sup>84</sup> When direct hyperbilirubinemia is present during the first 24 hours, especially in cord blood, it seems to be associated with very severe disease.<sup>122</sup> The pathogenesis of the "*inspissated bile syndrome*" is not known<sup>324</sup> and the term itself carries implications that may be incorrect.

Only unbound bilirubin is toxic to the central nervous system.<sup>119</sup> Indirect-reacting bilirubin readily associates with albumin in the circulation and therefore estimation of the binding capacity of albumin for bilirubin is of critical clinical importance. Since phenolphthalein (PSP) appears to compete with bilirubin for binding sites on albumin, the PSP binding capacity of serum may be used as a measure of the ability of albumin to bind additional bilirubin.<sup>132</sup> An inverse relationship between serum bilirubin levels and PSP binding capacity has been established, and this is used as an index of the need for exchange transfusions.<sup>133</sup> When the PSP technique is not available, the simple determination of albumin or total serum protein concentrations may be of value.

### Serologic Findings ✓

A positive reaction to the direct antiglobulin test given by cord blood erythrocytes is the characteristic finding in hemolytic disease of the newborn due to anti-Rh antibodies. The reaction is positive with anti-Ig sera only. Its strength may vary considerably, however, and occasionally the reaction may be negative when the test is performed by ordinary techniques, even when incomplete antibodies are found in the maternal serum.<sup>321</sup> On the other hand, the coating of red cells by antibody may sometimes be so heavy as to block the Rh antigenic sites and

interfere with Rh typing. In such cases, Rh positivity must be inferred from the strong reaction to the direct Coombs' test.<sup>122</sup> Without therapy the antiglobulin reaction may remain positive for several weeks, but will gradually become weaker; when infants are effectively treated with exchange transfusions, the reaction quickly becomes weak or negative.

Most infants with positive reactions in direct antiglobulin tests also have free antibodies in the serum at the time of birth.<sup>324</sup> These antibodies may be demonstrated by the indirect antiglobulin test.

### Antenatal Assessment of Severity

Proper prevention and therapy of HDN depend on the prenatal demonstration of biochemical, serologic, and clinical changes, both in mother and fetus.

### Serologic Changes in the Mother

Maternal Rh sensitization and HDN are rare during the first pregnancy, unless the mother has previously been sensitized by transfusion with Rh-positive blood. Antibodies are frequently detectable about six to eight weeks after the first sensitizing pregnancy, probably in response to the transfer of a large number of fetal cells into the maternal circulation at the time of delivery (page 896). Initially IgM saline reactive antibodies are produced that do not cross the placenta. Although these antibodies are of no direct significance to the fetus, even during pregnancy, they may herald the advent of IgG antibodies. These usually replace IgM antibodies if the antigenic stimulus is continued or renewed. Their concentration remains low, however, until a booster stimulus of fetal red cells leads to a further rise in antibody titer, usually during the third trimester of a subsequent pregnancy. Because of this pattern it has been found useful to obtain a baseline titer at about 16 weeks' gestation, a second determination at 28 to 32 weeks, and subsequent titers at intervals of one to four weeks, depending upon the rate of increase in anti-

body concentration.<sup>122</sup> Unfortunately, although there is a rough general correlation between maternal antibody titers and the severity of the hemolytic process in the infant,<sup>61,90,109,155,164</sup> especially during the first affected pregnancy,<sup>136</sup> exceptions to this general pattern do not allow complete reliance on maternal antibody titers alone.<sup>109,150</sup> Nevertheless, maternal antibody titers furnish a useful index of the baby's prognosis in the majority of patients.

The accuracy of predicting the outcome of a given pregnancy can be increased considerably if, in addition to measuring maternal antibody levels, the individual's family pattern, and particularly the severity of the disease in the first affected infant, is taken into account.<sup>150</sup> Chown<sup>95</sup> published data listing four grades of severity and the corresponding predictions of severity in the next affected child.

### Amniotic Fluid Examination

Amniotic fluid, which is normally colorless or the color of pale straw, may become bright yellow when severe HDN is present. The total composition of the pigment has not yet been determined but much of it appears to be bilirubin,<sup>104</sup> probably derived from fetal red cell heme. Neither is it known how the pigment enters the amniotic fluid from the plasma of the fetus.<sup>107</sup>

The total amount of amniotic pigment, as reflected by optical density measurement at 450 nm, correlates well with the severity of the anemia at birth,<sup>99</sup> as well as with the fetal outcome.<sup>39,100</sup>

The concentration of pigment in the amniotic fluid is usually measured by spectral photometry over the range of 350 to 700 nm (Fig. 27-2). The optical density of normal amniotic fluid describes a straight line over the range of 350 to 600 nm, but when pigment is present the so-called "bilirubin bulge" is seen with a peak at 450 to 460 nm.<sup>46</sup> The increased optical density at the peak can be estimated by joining the straight parts of the curve and determining the height of the bulge above the conjectural baseline. Impor-

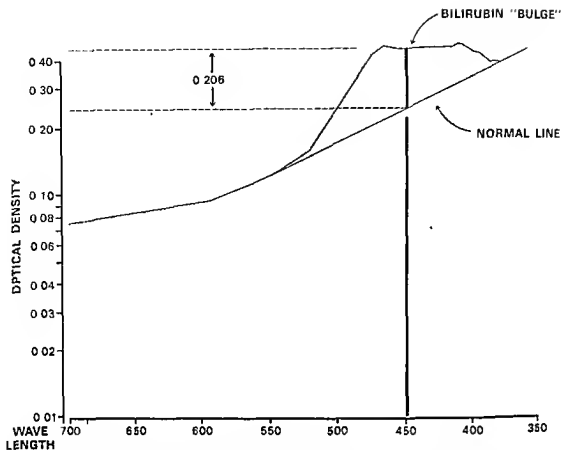


Fig. 27-2 Optical density curve of amniotic fluid obtained from an isosensitized woman. Normally a straight line is seen between 350 and 600 nm. The height of the "bilirubin bulge" is measured at 450 nm. The value obtained here (0.206) falls into zone 3 given a gestational age of 34.5 weeks (Fig. 27-3) (From Bowman and Pollock,<sup>46</sup> courtesy of the authors and Pediatrics.)

tant sources of error include (1) contamination with fetal blood, which may contribute bilirubin; (2) hemolyzed red cells of fetal or maternal origin that yield a false rise in optical density; (3) contamination with meconium or vernix caseosa that causes turbidity and interferes with optical density readings; and (4) exposure to light, which reduces pigment concentration by oxidation.<sup>122</sup> Since the optical density of normal as well as abnormal amniotic fluids tends to fall with advancing gestation,<sup>99</sup> it is important to interpret optical density data in the light of this knowledge. This is illustrated in Figure 27-3 in which the increase in optical density, corrected for gestational age, is roughly divided into three

zones indicating the approximate degree of fetal affliction.<sup>46</sup> A stationary or rising optical density measurement in repeated samples indicates worsening of the hemolytic process.

Although optical density measurements are most commonly employed in the examination of amniotic fluid, other techniques also have been recommended. Thus, amniotic fluid bilirubin levels may be measured biochemically, but since very low concentrations of bilirubin are often significant, special techniques are necessary.<sup>75,117</sup> Blood-stained specimens cannot be used for optical density measurements,<sup>120</sup> but if such samples contain fetal cells, the latter may be typed: if they are Rh negative, additional studies are unnecessary.<sup>47</sup>

The determination of bilirubin-protein ratios is useful,<sup>53,112,116</sup> since these are independent of the time of gestation and may also allow for variations in liquor amnii volume that may seriously affect pigment concentration. Indeed, the protein concentration of amniotic fluid is itself an excellent guide to the severity of the disease.

The concentration of anti-Rh<sub>0</sub> antibody in the amniotic fluid also appears to correlate well with the severity of the hemolytic process,<sup>72,135</sup> although this has been denied by some.<sup>72</sup> When used in conjunction with spectrophotometric techniques, it enhances considerably the accuracy of prediction.<sup>72</sup> The pathway by which the anti-Rh<sub>0</sub> enters the amniotic cavity is not known. When mothers with high concentrations of anti-Rh<sub>0</sub> carry a

Rhesus-negative infant, virtually no antibody appears in the amniotic fluid.<sup>72</sup>

*Technical details* of the collection, preparation, and examination of amniotic fluid are provided in several reviews.<sup>46,99,100</sup> When carried out by an experienced operator, the procedure seems to be fairly safe, and major complications are rare. Nevertheless, occasional cases of hemorrhage, including fetal exsanguination, and infection have been reported.<sup>122</sup> Placental trauma, with hemorrhage into the maternal circulation, appears to be a particularly common problem and may increase antibody production by the mother.<sup>165,168</sup> It is therefore most important to localize the placenta accurately before amniocentesis, either by ultrasound techniques or, if necessary, by isotopic methods.<sup>122</sup>

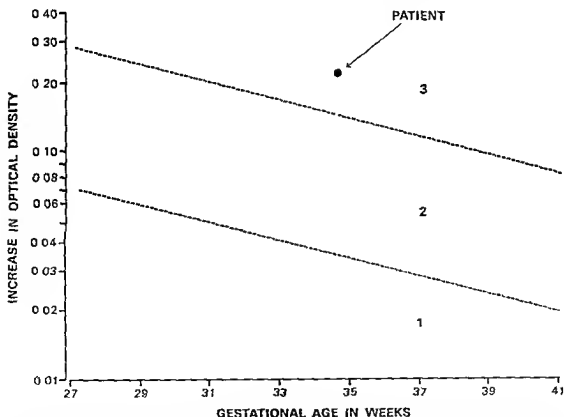


Fig. 27-3. Plotting the height of the bilirubin bulge (increased optical density at 450 nm—see Fig. 27-2) against gestational age. Three zones of severity are indicated: Zone 1, Rh-negative infant or mildly affected Rh-positive infant; Zone 2, intermediate disease; Zone 3, severe disease and impending fetal death. The value obtained from Figure 29-2 falls into Zone 3, given a gestational age of 34.5 weeks. (From Bowman and Pollock,<sup>46</sup> courtesy of the authors and Pediatrics.)

## Treatment

Optimal management of the sensitized mother and her Rh-positive infant requires continued antenatal and postnatal care of both infant and mother by a team of obstetricians, pediatricians, and serologists.<sup>122</sup> While antenatal care is focused on the diagnosis and therapy of severe anemia and hydrops fetalis, which constitute the two most important threats to fetal life, postnatal care is mainly concerned with the prevention of damage due to hyperbilirubinemia and severe anemia in the infant, and the suppression of anti-Rh antibody production in the mother.

## Prenatal Management

Proper antenatal care of the erythroblastic infant has to be based on considerations discussed in earlier sections, namely, (1) the birth order of the child; (2) maternal history in regard to the outcome of previous pregnancies, blood transfusions, etc.; (3) serologic tests including the maternal antibody titers and the father's Rh zygosity; and, finally, (4) the results of amniotic fluid examinations. On the basis of these considerations, three major courses of action are open to the obstetrician caring for an Rh-sensitized pregnant mother:

1. If the results of all tests and the historical considerations are favorable, the physician may decide to do nothing or to use controlled labor at 38 weeks.
2. If, on the other hand, there appears to be great risk of hydrops fetalis or stillbirth during the late stages of pregnancy, earlier induction between 34 and 38 weeks may be indicated.
3. Finally, if there is risk of hydrops fetalis or stillbirth before 34 weeks, intrauterine and intraperitoneal transfusions followed by induction of labor at 34 weeks become mandatory.

**EARLY INDUCTION OF LABOR.** When early delivery is not required for other reasons, routine "preterm" induction at about 38 weeks of gestation offers some undeniable advantages, including the ability to assemble

a well-prepared and coordinated team of obstetricians, pediatricians, and technical staff, an undertaking that may be more difficult during spontaneous, middle-of-the-night deliveries.<sup>122</sup>

In the more severely affected infants, premature delivery is induced at 34 weeks in order to decrease the incidence of stillbirths due to anemia, since about half the intrauterine deaths take place after the thirty-fourth to thirty-fifth weeks of pregnancy.<sup>35,153</sup> The decision to proceed with early induction is based primarily on the results of amniocentesis (above) and, to a lesser degree, on clues provided by the previous history<sup>56</sup> and antibody titers. A policy of premature induction of labor based on amniocentesis findings can lead to a drop in the stillbirth rate and a considerable drop in the neonatal death rate.<sup>102</sup>

**INTRAUTERINE TRANSFUSION.** At least half of the intrauterine deaths occur before the thirty-fourth week of gestation. Premature induction of labor cannot save these babies since the risk of death from causes related to prematurity is considerable, even in the absence of complicating diseases such as HDN: 15% at 34 weeks, 30% at 32 weeks, and 60% at 30 weeks.<sup>107</sup> When prematurity is complicated by HDN, the mortality rate must certainly be considerably higher.<sup>107</sup> The main threat to survival of these infants *in utero* is anemia. The success of intrauterine transfusions<sup>101,102</sup> depends on the fact that red cells introduced into the peritoneal cavity of the fetus find their way into the circulation through the thoracic duct and survive normally.<sup>102</sup> The technique should be undertaken only by an experienced obstetric and pediatric team.<sup>122</sup> Accurate localization of the placenta is mandatory. The volume and frequency of transfusion vary with the gestational age, 30 to 100 ml of packed red cells being administered every two to three weeks until the baby is delivered at 34 to 35 weeks of gestation. With successful transfusions the amount of adult hemoglobin in cord blood should be of the order of 55 to 95% instead

of the usual proportion of 15 to 40%.<sup>122</sup> Complications include trauma to the fetus, trauma to the placenta, infection in mother and fetus, and onset of premature labor.<sup>148</sup> Two cases of suspected graft-versus-host disease have also been described and are presumably attributable to the transfer of incompatible lymphocytes into an immunologically immature recipient.<sup>148</sup>

While some babies are undoubtedly saved by intrauterine transfusions, the overall results are discouraging: stillbirths still occur in 48 to 55% of those thus treated and 13 to 32% of those born alive die in the neonatal period, leaving an overall survival rate of only 35 to 40%.<sup>148</sup> Complications in live-born babies include unexplained reticulocytopenia, an unusually high incidence of the "inspissated bile syndrome," and hypoglycemia. In addition, the neonatal death rate may be seven times as high as that of infants with hemolytic disease who are delivered spontaneously.

### Postnatal Management

**EXCHANGE TRANSFUSIONS.** The first exchange transfusion for HDN was performed in Canada in 1925,<sup>81</sup> but no attention was paid to this novel approach to therapy until almost 20 years later when a similar procedure was popularized by Wallerstein,<sup>149</sup> Wiener,<sup>154</sup> Diamond,<sup>63</sup> and Mollison,<sup>108</sup> among others. The results of exchange transfusions since then have been extremely gratifying and the mortality of live-born affected infants has been greatly reduced as a direct result of this therapy.<sup>36,107,110,324</sup>

The objectives of exchange transfusions include: (1) the removal of antibody-coated red cells from the circulation of the infant; (2) the correction of anemia and the reversal of congestive failure in hydropic or prehydropic infants; and, later, (3) the removal of bilirubin.

Since about 85% of antibody-coated red cells may be removed by the initial exchange transfusion, the number of red cells at risk can be reduced considerably and the subse-

quent production of bilirubin is greatly curtailed (each gram of hemoglobin from hemolyzed red cells yields 35 mg of bilirubin, Chapter 5). Thus, an exchange transfusion carried out within a few hours of birth not only forestalls the development of severe anemia but also greatly reduces the chance of kernicterus.<sup>107</sup>

Infants born with severe anemia are almost always in cardiac failure. While these infants urgently require red cells, simple transfusion is very dangerous since it inevitably makes congestive failure worse. Exchange transfusion, during which a greater volume of blood is removed than is injected, is the only safe alternative. In these infants, packed red cells are used instead of whole blood. The venous pressure is monitored throughout the procedure by measuring the level to which the blood rises in the umbilical vein catheter.<sup>63</sup>

One complete exchange transfusion (200 ml/kg) also removes about 90% of the plasma bilirubin. However, the extravascular pool of this pigment is much greater than the intravascular pool, and reequilibration occurs within 30 minutes.<sup>140</sup> This accounts for the "rebound hyperbilirubinemia" noted after the exchange, with final plasma concentrations of 40 to 60% of the initial level.<sup>49,140</sup> Because extravascular and intravascular bilirubin equilibrate rapidly, the final size of the bilirubin pool depends, at least in part, on the length of time taken to make the exchange, which should probably never be less than 90 minutes.<sup>107</sup>

Indications for exchange transfusions vary widely among different workers. The information needed and the criteria that are applied in arriving at a reasonable decision are summarized in Table 27-4. In addition, a graph of serum bilirubin levels plotted against the infant's age, such as that prepared by Allen and Diamond,<sup>122</sup> is helpful in anticipating the need for further exchange transfusions in the hyperbilirubinemic infant.

The technique of exchange transfusion has been described.<sup>107,120,122</sup> The umbilical vein is the most preferable route of exchange. One hundred and sixty to 200 ml/kg is exchanged

**Table 27-4. Need for Exchange Transfusion in Infants with a Positive Coombs' Reaction—Suggested Course of Action**

<i>Findings</i>	<i>Observe</i>	<i>Consider Exchange</i>	<i>Do Exchange</i>
<b>At Birth</b>			
History or course of action in previous offspring was	No exchange transfusion	Exchange transfusion was necessary or kernicterus was observed	Death or near death from erythroblastosis
Maternal RH antibody titer	< 1:64	> 1:64	
Clinical situation	Apparently normal	Induced or spontaneous delivery of premature infant	Jaundice, fetal hydrops
Cord hemoglobin	> 14 g/dl	12–14 g/dl	< 12 g/dl
Cord bilirubin	< 4 mg/dl	4–5 mg/dl	> 5 mg/dl
<b>After Birth</b>			
Capillary blood hemoglobin	> 12 g/dl	< 12 g/dl	< 12 g/dl and falling in first 24 hours
Serum bilirubin	< 18 mg/dl	18–20 mg/dl	20 mg/dl in first 48 hours or 22 mg/dl on two successive determinations at 8 to 8-hour intervals after 48 hours
			Clinical signs suggesting kernicterus at any time or at any bilirubin level

From McKay<sup>104</sup> courtesy of the author and Pediatrics

over a period of at least 90 minutes. Heparinized blood is preferable because of the potential complications of the acid citrate dextrose anticoagulant, although the latter is usually more readily available. The blood should be fresh in order to assure high 2,3DPG levels in the red cells (Chapter 11) and in order to minimize the dangers of hyperkalemia and acidosis inherent in the use of older blood. It should be O Rh negative and should always be cross-matched against the mother's serum by the indirect Coombs' test, preferably prior to delivery. When indicated, the cross-match procedure should include a sickle cell test (Chapter 25) of the donor blood.

Since damage to the central nervous system is primarily attributable to free indirect-reacting bilirubin, that is, pigment unbound to albumin, it has been found advantageous to add albumin to the exchange transfusion,

particularly when given to severely jaundiced babies.<sup>120,153</sup> The usual dose is 1 g/kg. Albumin is *not* to be used in infants with hydrops fetalis. It has been suggested that phototherapy may reduce the requirements for exchange transfusion in some patients.<sup>129</sup>

*Complications*<sup>122</sup> include (1) the *biochemical changes* of hyperkalemia due to the use of old blood; hypocalcemia, increased blood citrate levels, and acidosis due to the use of ACD stored blood; (2) the *cardiovascular complications* of air or blood-clot emboli, septic thrombosis, volume overload, and cardiac arrest, the latter primarily due to factors listed under (1); (3) *clotting defects*, especially those due to overheparinization or thrombocytopenia<sup>52,107</sup>; and (4) *bacteremia and serum hepatitis*.<sup>89a</sup> The mortality rate directly attributable to exchange transfusion should be less than 1%.<sup>107</sup>



## Prevention of Rh Hemolytic Disease

The most effective means of protecting infants against Rh hemolytic disease are those that prevent maternal sensitization to fetal Rh antigens or inhibit the production of antibodies specific for those antigens. Two methods are available<sup>165,166</sup>: (1) by preventing large transplacental hemorrhages that expose patients to the high risk of immunization, and (2) by passive immunization of Rh-negative mothers with anti-Rh antibodies. The former can be accomplished by meticulous obstetric care and particularly by the elimination of maneuvers that disturb the chorionic site, including cesarean section, manual expression of the placenta, and needling of the placenta during amniocentesis.

A concept for the prevention of Rh immunization by passive administration of Rh antibodies<sup>70,73</sup> evolved from the observation that (1) immune responses could be blunted considerably by the concomitant administration of specific antibodies<sup>134,138</sup> and (2) Rh immunization rarely resulted from a pregnancy in which the fetal erythrocytes were ABO incompatible with the mother's serum.<sup>141</sup> The hypothesis was first tested in Rh-negative volunteers who received Rh-positive blood by injection.<sup>159,165</sup> It was clearly shown that the concomitant administration of anti-Rh antibodies could bring about a ten-fold reduction in the incidence of sensitization (three out of 78 vs 29 out of 75).<sup>165</sup> Similarly spectacular results were subsequently achieved in well-controlled studies<sup>125</sup> in which the incidence of sensitization in high-risk women who received anti-Rh gamma globulin within 72 hours postpartum was compared with that of high-risk women who received no such therapy—none of 75 antibody-protected women developed antibodies, whereas 19 of 78 unprotected mothers did. Clinical trials involving *all* women at risk confirmed these findings.<sup>57,67,159,162,166</sup> In addition to the lack of antibodies six months postpartum, the incidence of antibody production during the

next pregnancy also was greatly reduced.<sup>57</sup> Indeed the point has been made that protection must not be considered complete until these women have gone through another pregnancy and have remained free of antibodies.<sup>165</sup>

Although the risks of immunization with *abortion* may be less than that associated with normal termination of pregnancy<sup>44,115,159</sup> they are nevertheless considerable.<sup>45,74,159,166</sup> It is therefore advisable to give Rh prophylaxis to *all* women who abort unless the sire is known to be Rh negative.

*Standard prophylaxis* consists of 300 µg of anti-Rh<sub>0</sub> (D) antibody given intramuscularly to the unsensitized Rh-negative woman within 72 hours of delivery of an Rh-positive infant. Some European centers have given smaller amounts of a more highly purified preparation intravenously, with excellent results.<sup>44</sup> Indeed, much faster elimination of Rh-positive cells from the maternal circulation<sup>42,159</sup> and a much lower incidence of failures (one out of 3,695) have been reported with intravenous prophylaxis than with intramuscular.<sup>159</sup> If these studies can be confirmed, the intravenous route of antibody administration may find much wider application in the future.

Standard prophylaxis may be insufficient to deal with *massive* transfusions of Rh-positive cells of either fetal or donor origin.<sup>44,159,166</sup> Since the number of women experiencing a major (>30 ml) transplacental hemorrhage may approach 1%,<sup>166</sup> routine screening procedures for the detection of large amounts of bleeding are necessary. Doses of 10 to 25 µg anti-Rh<sub>0</sub> antibody/ml of Rh-positive red cells in the maternal circulation have been recommended.<sup>44</sup> Clearance and protection studies carried out in male volunteers suggest that these doses probably are adequate. Such large quantities of gamma globulin should always be given intramuscularly.

When adequate doses of antibodies are used, protection against Rh immunization is *virtually* complete.<sup>166</sup> A small number of primigravidae (1.7%<sup>56</sup>) may develop antibodies *during* their first pregnancy, however,

and under these circumstances postpartum prophylaxis would be useless.<sup>168</sup> Whether such sensitization can be prevented by the routine use of anti-Rh globulin antepartum remains to be seen. Since IgG antibodies readily cross the placenta, the administration of anti-Rh globulin to a pregnant woman is not without potential risk to the fetus, but to date no harm has come to babies whose mothers were so treated.<sup>41,169</sup>

Rh-immune globulins are produced from the plasma of artificially immunized male volunteers or from highly sensitized Rh-negative women who may be hyper-immunized by injection of Rh-positive cells if they can no longer bear children.<sup>41</sup> Many produce antibody levels in excess of 100  $\mu\text{g}/\text{ml}$  serum, although levels in the range of 30 to 100  $\mu\text{g}/\text{ml}$  are more common. The sera are usually obtained by weekly plasmapheresis (600 to 700 ml) of these donors without apparent harm over prolonged periods.

The way in which anti-Rh antibody brings about specific suppression of the anti-Rh immune response is still under active investigation. To date, three main possibilities have been considered,<sup>159</sup> namely, (1) that immunosuppression is due to the destruction of the antigen, or its shunting, from sites where sensitization could take place to areas in the reticuloendothelial system that are immunologically naive (this possibility is exemplified, for instance, by the protection afforded by ABO incompatibility); (2) that it is due to the blocking or binding of antigenic determinants so that effective contact with antigen-sensitive cells is prevented; or (3) that it is due to a direct suppressive effect of antibody on the antigen-sensitive cells themselves.

## Hemolytic Disease of the Newborn (HDN) Due to ABO Incompatibility

### Pathogenesis

Hemolytic disease of the newborn due to

ABO incompatibility results from the interaction of maternal anti-A or anti-B antibodies with fetal erythrocytes carrying the corresponding blood group antigens. While HDN due to ABO incompatibility is about twice as common as that due to Rh incompatibility, it is rarely as severe and often goes unnoticed, its mild clinical manifestations blending deceptively into the bilirubin-tainted landscape of neonatal physiology.

Statistically, about 20% of all pregnancies involve ABO incompatibilities of the type that could lead to HDN,<sup>37</sup> but the incidence of significant hemolytic disease is only one in 150 births, and about one in five babies at risk develop jaundice.<sup>107</sup> Moreover, while several patterns of maternal-fetal ABO incompatibilities are possible, virtually all hemolytic disease occurs in A or B infants of group O mothers. This curious phenomenon is attributable to the nature of the anti-A or anti-B antibodies: only group O mothers produce sufficient quantities of IgG anti-A or anti-B antibodies, the corresponding antibodies of group A or B mothers being confined to the IgM variety which cannot cross the placental barrier (page 312).

Since group O individuals are "naturally" presensitized to A and B antigens by exposure to ABO-like substances found in food and other exogenous sources, first-born infants are affected as frequently as those born subsequently. Indeed, even when the first infant has suffered from HDN, other incompatible siblings may or may not have the disease, there being no predictable increase in the severity of the hemolytic process in succeeding siblings as occurs in Rh hemolytic disease.

The ability of secretor (Chapter 11) infants to produce soluble blood group substances does not appear to protect them against ABO hemolytic disease.<sup>107</sup> This may be due to the fact that IgG antibodies are more difficult to neutralize by soluble blood group substances than are IgM antibodies.<sup>92</sup> Indeed, the ratio of secretor to nonsecretor babies is slightly higher than expected,<sup>143</sup> leading to the suggestion that the secreted blood group substances may play a role in sensitization.

## Clinical Manifestations

The most common manifestation of ABO hemolytic disease is jaundice. Like that of Rh hemolytic disease, it appears during the first 24 hours of life but is not as pronounced; it very rarely is sufficiently severe to cause complications such as kernicterus. The anemia is correspondingly mild, pallor is uncommon, and hydrops fetalis is exceedingly rare. Mild degrees of hepatosplenomegaly may be observed.

## Laboratory Findings

In keeping with the clinical manifestations, the hemoglobin is usually normal, but on occasion it may drop as low as 10 g/dl. The blood smear shows evidence of compensatory erythropoiesis with polychromatophilia and a few nucleated red cells. The reticulocyte count is appropriately elevated. Spherocytes, which are not seen in Rh hemolytic disease, may be a striking feature of the peripheral smear.

Elevated serum bilirubin values frequently constitute the only laboratory evidence of hemolysis, and, as in Rh hemolytic disease, the indirect-reacting fraction is predominantly elevated (page 900).

## Serologic Findings

The infant's red cells often give a negative or only weakly positive reaction to the direct antiglobulin test, even though A or B specific antibodies, which interact strongly with fetal or adult cells *in vitro*,<sup>82,143</sup> can be eluted.<sup>60</sup> This failure of antibody-sensitized fetal cells to agglutinate with antiglobulin sera may be a function of the smaller number of antibody molecules sensitizing these fetal cells<sup>131</sup> which in turn may, at least partially, reflect the greater distance between sites in fetal cells as compared to the distance in adult cells.<sup>144</sup>

Various modified techniques have been devised in an effort to improve the detection of sensitized cells.<sup>107</sup> While some give better results than others, none gives the strong, reliable antiglobulin reaction seen with Rh hemolytic disease.

Free antibody against adult cells frequently

is demonstrable in the infant's serum and, when it has specificity for the infant's A or B antigen, the presence of ABO hemolytic disease must be presumed. Reactions to the *indirect* Coombs' test were found to be positive in 39 of 42 jaundiced ABO-incompatible infants when the tests were performed during the first 24 hours following birth.<sup>79</sup> Complement is not found on the red cells of babies suffering from ABO hemolytic disease<sup>151</sup> and the complement titers of these babies are normal,<sup>151</sup> suggesting that the mechanism of hemolysis is complement independent and perhaps akin to that of Rh HDN (page 894).

## Antibodies in the Maternal Serum

In some ABO-incompatible pregnancies, other immune properties are found in mothers' serum, including the presence of hemolysins and agglutinins that are difficult to neutralize with A and B substances, and that react with group A pig cells.<sup>71,92,107</sup> However, only one of three infants born of mothers with such antibodies shows hemolytic disease. Since only non-inhibitable 7S antibodies are associated with disease, a better correlation between the presence of antibodies and hemolytic disease could be expected if IgG antibodies were separated from the IgM antibodies. A relatively simple test that seems to fulfill these requirements has been devised<sup>124</sup>; a high titer ( $> 1000$ ) was found in 13 of 18 infants requiring exchange transfusions, in four of 50 who did not require exchange transfusions, and in one of 16 healthy, incompatible infants.

## Treatment

Severe anemia is very uncommon and therapy, when necessary, is therefore directed toward control of hyperbilirubinemia. Phototherapy is very useful for this purpose.<sup>89,133</sup> Exchange transfusion with whole blood should be carried out when the bilirubin level threatens to exceed 20 mg/dl. Group O blood of the infant's Rh type should be used, although O cells suspended in AB plasma would theoretically be preferable.<sup>107</sup> Some authors, however, do not consider the use of

AB plasma worthwhile.<sup>77</sup> If the use of AB plasma is not practicable, donors with low anti-A or anti-B hemolysin titers should be used. The principles and methods of exchange transfusion are otherwise the same as those for Rh hemolytic disease.

## Immuno-hemolytic Anemias Due to Warm Reactive Antibodies

Immuno-hemolytic anemias due to warm reactive autoantibodies (*autoimmune hemolytic anemias*, AIHA) readily fall into three broad categories<sup>217</sup>: (1) those in which the hemolytic anemia dominates the clinical picture and seems to be unaccompanied by other coexisting or underlying disease ("idiopathic" or "primary"); (2) those in which the hemolytic process is closely linked to some other well-defined disease ("symptomatic" or "secondary"); and (3) those immuno-hemolytic anemias clearly associated with exposure to drugs or chemicals. The first category is to be regarded as a convenient temporary grouping and will eventually be eliminated through increased understanding of basic pathogenetic mechanisms. For purposes of this discussion it is convenient to deal with the first two categories as a unit; the drug-induced hemolytic anemias will be considered separately below. The diagnostic approach to the patient with hemolytic anemia is discussed in Chapter 20 (page 737).

### Etiology and Pathogenesis

Warm reactive antibodies with specificity for red cell antigens have been described in association with a number of diseases (Table 27-5), including viral infections, malignant conditions, immune deficiency states, and as part of other diseases with "autoimmune"

**Table 27-5. Autoimmune Hemolytic Anemias (AIHA)**

<b>I. Warm reactive antibodies</b>	
<input checked="" type="checkbox"/> A	Primary or idiopathic, without obvious cause
<input checked="" type="checkbox"/> B	Secondary or symptomatic, associated with
1	Chronic lymphocytic leukemia
2	Lymphoma
3	Non-lymphoreticular tumors
4	Autoimmune diseases, especially lupus erythematosus
5	Viral infections
<b>C</b> Drug-dependent antibodies	
1	Penicillin type
2	Stribophen type
3	Alpha methyl dopa type
<b>II. Cold reactive antibodies</b>	
<input checked="" type="checkbox"/> A	Primary cold agglutinin disease
<input checked="" type="checkbox"/> B	Secondary cold agglutinin disease, associated with
1.	Infections: M. pneumoniae, infectious mononucleosis
2	Lymphoreticular neoplasms
<input checked="" type="checkbox"/> C.	Paroxysmal cold hemoglobinuria
1	Idiopathic
2	Secondary
a	Syphilis
b.	Viral infections, especially measles, mumps

features, such as systemic lupus erythematosus. Many theories have been proposed to explain the production of antierythrocyte antibodies under such circumstances. These include (1) alterations in the patient's erythrocytes that make surface antigens no longer recognizable as self; (2) disturbances of immune responsiveness, including the emergence of forbidden clones; and (3) genetically determined peculiarities of immune responsiveness that lead to cross-reactivity of normal immune responses with the patient's own tissues.

Although erythrocyte surfaces can be damaged by viruses in vitro and new antigens can be uncovered by enzymatic damage to red cells,<sup>217</sup> there is insufficient clinical evidence to suggest that this actually occurs in vivo or that human erythrocytes damaged in some way become antigenic. In this context it is particularly important to emphasize that many of the red cell autoantibodies actually interact with *normal* antigenic determinants,

\*"Autoimmune" is used as a convenient descriptive term only. It signifies the presence of immunologic processes with specificity for antigens found on the tissues of the individual making the immune response. The term is not intended to convey any implication as to mechanisms, as, for example, the emergence of "forbidden clones."

particularly those belonging to the Rh group of antigens.

The concept of autoimmunity as the result of disturbed immune mechanisms implies a loss of fetally acquired tolerance towards erythrocyte autoantigens. This fundamental defect is thought to develop in immunologically competent cells as a result of somatic mutation<sup>210,211,219</sup> with the emergence of new clones of antibody-producing cells that no longer recognize certain antigens as "self." This concept is thought to derive support from the association of autoimmune hemolytic anemias with recognizable disorders of the lymphoid system and with diseases having other autoimmune manifestations.<sup>286</sup> It should be pointed out, however, that such associated disorders could equally well have arisen as the result of some other cause common to both disorders, such as viral infection. Indeed, there is no direct evidence to support the contention that autoimmune disease ever results from specifically acquired derangements in immune responsiveness.

Occasionally, autoimmunity seems to be the result of a normal immune response toward a foreign antigenic determinant that cross-reacts with an autologous tissue constituent. This has been observed, for example, in a patient suffering from chronic phenacetin ingestion; in time this patient produced anti-phenacetin antibodies that also reacted against an autoantibody found on the patient's own red cells.<sup>246</sup> Similarly some strains of mycoplasma are thought to have antigenic determinants similar to the I antigen of human red cells,<sup>285,386</sup> resulting in the formation of cold agglutinins cross-reacting with all erythrocytes carrying this antigen. Although this claim has not been confirmed by others,<sup>235</sup> sufficient evidence of cross-reactivity exists in other forms of autoimmune disease<sup>300</sup> to maintain it as an attractive hypothesis.

In some autoimmune phenomena the destruction of autologous tissues may simply be the result of close proximity between a foreign antigenic determinant and normal tissues caught in the immune attack as "innocent bystanders." This mechanism has been docu-

mented in some drug-induced immunohemolytic anemias (see below), in certain "slow-virus" infections,<sup>262,274</sup> and in graft-versus-host disease.<sup>231</sup>

One of the most promising concepts of autoimmunity is derived from animal studies that link genetic factors and persistent viral infections, on the one hand, with autoimmune phenomena, including Coombs'-positive hemolytic anemia, on the other.<sup>262</sup> NZB mice appear to pass murine leukemia virus vertically from generation to generation and seem incapable of eliminating the virus until late in the lifetime of infected animals.<sup>207,262</sup> By midlife, virtually all mice of this strain have Coombs'-positive hemolytic anemia, the majority have membranous glomerulonephritis, and some develop malignant lymphomas and sarcomas. It is particularly noteworthy that the development of a positive reaction to the Coombs' test in NZB mice corresponds closely in time to the production of specific virus-derived soluble antigen. Immunologic maneuvers that inhibit the appearance of soluble antigen also inhibit the appearance of Coombs' positivity, whereas manipulations that aid the early proliferation of the virus also accelerate the appearance of anti-red cell antibodies.<sup>207,262</sup> Antibodies eluted from affected red cells interact equally well with normal mouse erythrocytes irrespective of strain,<sup>257</sup> suggesting that the antibodies are not directed against virus-modified red cell antigens adhering to the red cell surface. These findings strongly suggest a direct link between leukemia-like viral infection and the development of immune hemolytic anemia in NZB mice. They may also illuminate several observations made in regard to human immunohemolytic disease; eg, the frequent association of "idiopathic" Coombs'-positive hemolytic anemias with immune deficiency states, both general and restricted (Chapter 44), thymic disorders,<sup>263</sup> chronic virus infections,<sup>301</sup> lymphomas,<sup>277</sup> and various other autoimmune phenomena,<sup>219,286</sup> including systemic lupus erythematosus. It is also noteworthy that the development of autoimmune manifestations in NZB mice appears to be under some sort of genetic control.

## Incidence

Autoimmune hemolytic anemias are not confined to any particular race although most published reports have dealt with Caucasian patients.<sup>217</sup> While it has generally been thought that there is no genetic basis for the disease, recent observations in man<sup>237,278,295</sup> and animals<sup>260</sup> suggest that a reevaluation of these views may be worthwhile (see also page 911). An increasing number of human cases of autoimmune hemolytic anemia in which a familial incidence is clearly demonstrable are being reported.<sup>219</sup> The disease is found in members of both sexes, but an excess of cases in females has been reported, particularly the "idiopathic" type of disease.<sup>219</sup> Subjects of all ages are affected. There seems to be an increased number of cases of "symptomatic" or "secondary" autoimmune hemolytic anemia of the warm antibody type in patients over 45, whereas the number of idiopathic cases is fairly evenly distributed throughout life.<sup>219</sup>

## Clinical Manifestations

The onset and course of autoimmune hemolytic anemia (AIHA) of the warm antibody type are most variable; the hemolytic process may be so mild as to be barely discernible by erythrocyte survival studies or it may be fulminant and life-threatening. Hemolysis of gradual and insidious onset frequently is seen in association with malignant disease or lupus erythematosus, whereas explosive and life-threatening hemolytic episodes seem particularly common when autoimmune hemolysis accompanies acute viral infections or when the underlying cause is obscure, especially in children.<sup>217</sup> The duration of the disease also varies considerably; although occasionally the episodes are short-lived, especially when hemolysis complicates acute infectious processes, the course is more commonly chronic and intractable. When the autoimmune hemolysis accompanies disorders such as Hodgkin's disease or other lymphomas the hemolytic process may wax or wane with the underlying disease.

Symptoms are most commonly related to the anemia and in patients with mild or chronic disease may include undue fatigue, dyspnea on exertion, and palpitation. When the hemolytic process is acute and massive the anemia may be so fulminantly progressive as to lead to shock-like prostration and semi-coma.<sup>217</sup> Sometimes jaundice is the patient's first complaint, but this is a variable symptom. When the disease is of acute onset and very severe, hemoglobinuria may be noted. Unexplained fever and abdominal pain occur often enough to be selectively noted.<sup>245</sup> Other less common symptoms include thrombophlebitis, precordial pain, and headache.<sup>217</sup> Additional symptoms may point to underlying disease. The history should always include detailed information concerning the drugs taken by the patient (see page 916). Pertinent physical findings include pallor and jaundice, which in severe cases may be accompanied by a peculiar cyanosis of the lips, nose, cheeks, and ears, presumably because of vascular stasis brought about by intravascular autoagglutination. Splenomegaly is moderate and is found in about two thirds of all of these patients; there may also be mild to moderate hepatomegaly, especially in the more severe cases. If the autoimmune hemolytic disease is secondary, physical findings include those associated with the underlying disease.

## Laboratory Findings

### Blood

The hematocrit and hemoglobin values vary, depending on the severity of the hemolytic process. The MCV is usually in the macrocytic range, reflecting a young population of cells. The absolute reticulocyte count is correspondingly high and may reach well over  $1000 \times 10^9/l$ , but concurrent infection with marrow shutdown or infiltration of the marrow by malignant cells may markedly reduce the degree of reticulocytosis. The blood will show regenerative macrocytosis, with considerable polychromatophilia and anisocytosis, often with a striking number of

microspherocytes. Occasionally, thin projections from the surface of red cells are seen. Nucleated red cells including macronormoblasts are frequently observed. Autoagglutination is a more characteristic feature of "complete" antibodies (page 924) but is seen with "incomplete" antibodies, particularly when the erythrocytes are strongly sensitized. When present, autoagglutination must be distinguished from rouleaux formation (page 1611). The white cell count may vary from that of a moderate leukopenia to that of a moderate leukocytosis; the platelet count usually gives normal values. Occasionally thrombocytopenia is noted and in some instances it is thought to be due to anti-platelet antibodies (see Evans' syndrome, page 1095).

The serum bilirubin level is moderately increased (usually 2.5 to 5 mg/dl) with a predominance of indirect-reacting pigment. When hemolysis is severe haptoglobin levels may be low or absent in spite of the predominantly extravascular destruction of the red cells; the plasma hemoglobin value usually is slightly elevated. Occasionally, massive hemoglobinemia, hemoglobinuria, and hemosiderinuria are seen, usually in patients with acute fulminant disease. In these subjects, methemalbumin may also be demonstrable spectroscopically. Mild forms of hemosiderinuria are not unusual in patients with chronic hemolysis and may indeed lead to iron-deficiency anemia.<sup>245</sup> Urine and fecal urobilinogen levels are correspondingly elevated and the latter may reach levels of over 1000 mg/day. The osmotic fragility usually is increased<sup>217</sup> in direct proportion to the number of spherocytes seen on the peripheral smear.

### Serologic Findings

When a positive reaction to the direct anti-globulin test is obtained in patients with hemolytic anemia, this constitutes strong evidence for an autoimmune or isoimmune etiology. Only rarely does a positive reaction occur in healthy persons<sup>295</sup> or in patients with nonimmune hemolytic anemias.<sup>217</sup> It is useful to determine the specificity of the serologic reaction. Initially, antiglobulin tests were

carried out with antisera prepared by immunizing animals with whole human serum. Naturally these reagents detected the presence of all gamma globulin classes, complement, and many other serum proteins. Most laboratories now use antisera that specifically react with IgG, complement, or one of the other immunoglobulin classes. In one representative study<sup>219</sup> of 43 patients with idiopathic or secondary autoimmune hemolytic anemia, 21 were found to react with anti-IgG only, 12 reacted with anti-IgG and anti-complement sera, and 7 reacted with anti-complement reagents only. A small number of patients ( $\frac{3}{43}$ ) also reacted with anti-IgA or anti-IgM sera. Positive reactions to complement specific Coombs' tests are particularly common in patients with systemic lupus erythematosus.<sup>267</sup>

Autoantibodies usually can also be demonstrated in the patient's serum when the reaction to the direct Coombs' test is positive and the patient is actively hemolyzing.<sup>219</sup> Methods using enzyme-treated cells are more sensitive and often reveal antibodies that cannot be demonstrated otherwise.<sup>217</sup>

ACQUIRED HEMOLYTIC ANEMIA WITHOUT DETECTED ANTIBODY. Occasionally, patients are encountered who have all the clinical manifestations of AIHA but in whom anti-red cell antibodies cannot be demonstrated by routine techniques.<sup>217</sup> Such patients may nevertheless destroy large volumes of transfused normal blood at rapid rates approximating the destruction of their own cells.<sup>233</sup> In some of these patients, plasma factors that promote the phagocytosis of red cells are found.<sup>275</sup> In others, it is possible to detect the presence of small quantities of anti-red cell antibodies by the more sensitive "complement-fixing antibody consumption technique,"<sup>249</sup> in which the patient's red cells and normal red cells are compared for their capacity to absorb anti-IgG from aliquots of specific rabbit antiserum. The reduction in anti-IgG antibodies is quantitated by a complement fixation test and the number of IgG molecules per red cell can then be derived from a standard curve. The number of IgG

molecules per sensitized red cell so detected is small (70 to 434 molecules per cell), but the serologic behavior of these antibodies appears to be similar to that of the traditional IgG antibodies of AIHA.<sup>210</sup> Why such small amounts of antibody should lead to overt hemolytic anemia is not clear, especially when one contrasts these patients with "Coombs'-positive normal people"<sup>295</sup> and with certain patients with strongly positive reactions to direct antiglobulin tests who only display the mildest hemolytic state.

With sensitive techniques it can be shown that the antibodies of about 70% of all patients with AIHA have specificity for some part of the Rh substance.<sup>296</sup> With rare exceptions<sup>213</sup> these antibodies will not react with Rh-null cells, which lack all antigenic components of the Rh complex (see Chapter 11); this suggests that the reacting antigen is some basic structural feature of the Rh complex. Most but not all<sup>208, 256</sup> of these antibodies are "less specific" than the isoantibodies developing after immunization with Rh antigens during pregnancy or transfusion<sup>219</sup>; on the other hand, this may simply be a reflection of the multispecificity of the antibodies in autoimmune hemolytic anemia.

When gamma globulins are eluted from sensitized red cells and their heavy chains are examined, they are found to be structurally heterogeneous.<sup>201, 268</sup> Occasionally, however, only one type of light chain is encountered and, when present, it is usually kappa.

## Treatment

When the diagnosis of AIHA is first made it is important to search for possible associated disorders such as tumors, lymphomas, and collagen vascular diseases (Table 27-5), since their therapy may take precedence over treatment of the hemolytic process as such. Indeed, effective therapy of the patient with a tumor or lymphoma may be accompanied by a remission of the hemolytic disease. Often, however, it is necessary to treat the hemolytic anemia as a separate entity by measures outlined below.<sup>253</sup>

*Blood transfusion* may be necessary as an

emergency measure, especially in patients with severe and fulminant disease, but its usefulness depends on the availability of more specific therapy, since the transfused blood is usually destroyed as rapidly as the blood of the patient. Occasionally, when the antibody has specificity for a well-defined red cell antigen, it may be possible to select blood lacking that particular determinant.<sup>298</sup> Usually, however, the specific antibody is accompanied by other "nonspecific" ones. In addition, it is frequently impossible to select blood not containing the specific antigen without also deliberately sensitizing the patient to other red cell antigens.<sup>298</sup>

*Blood typing and matching* may present special problems in AIHA. The patient's own erythrocytes may be difficult to type because of "blocking" of antigenic sites by antibody.<sup>217</sup> Under these circumstances it is sometimes possible to elute the offending antibody by heating the red cells to 56° C for 10 minutes.<sup>217</sup> More commonly, "nonspecific" antibodies found in the patient's serum make proper matching impossible because they appear to react with all donor cells in the indirect antiglobulin test. In such cases, it frequently is necessary to test as many donor samples as possible, and to select those whose red cells seem to give the best match. Such blood should only be used when absolutely necessary and must be administered very slowly and under constant supervision. Sometimes the use of such poorly matched blood is made relatively safe by the concomitant use of steroids (see below).

*The adrenocorticosteroid hormones* now constitute the initial therapy of choice in most patients with AIHA of the "incomplete" warm antibody type. Appropriate doses are those that achieve the desired clinical result; 40 mg/m<sup>2</sup> of body surface per day is a reasonable initial dose, but twice this amount or more may occasionally be needed. While the response to therapy is sometimes dramatic, hematologic improvement may not become evident before the third or fourth day, but it should always be evident within a week. There may be a transient increase in reticulocytes, and a weekly increment of 2 or



3 g of hemoglobin/dl of blood should occur. When the hemoglobin level has reached 10 g/dl or more, the therapeutic dose may be decreased gradually, the rate and degree depending on the hematologic response. After two or three weeks the indirect Coombs' reaction may become negative, but many patients undergo a remission with no demonstrable serologic change.

It is difficult to cite entirely satisfactory statistics for the effectiveness of adrenocorticoids in the treatment of patients with AIHA due to warm reactive, incomplete antibodies. It has been stated that complete relief from anemia can be expected in 70 to 90% of subjects,<sup>200,225,226</sup> with poorer results in those having the symptomatic forms. In perhaps a third of the patients with the idiopathic variety the remission will be sustained on complete withdrawal of therapy. In others, maintenance therapy in amounts of 5 to 20 mg prednisone per day may be required for many months and even years. Whatever may be the nature of the underlying cause in the patient achieving complete remission, it is likely that steroid therapy has simply made it possible for that patient to survive until spontaneous recovery has taken place. The ill effects of long-term treatment with steroids must always be borne in mind; these include increased susceptibility to infection, peptic ulceration, steroid myopathy, osteoporosis, hypertension, and diabetes. When large doses are used, salt restriction and the administration of potassium salts will usually be required. Insofar as possible, adrenocorticoid therapy should be held to the minimum dose compatible with satisfactory control of the hemolysis.

Steroids may ameliorate hemolysis in several ways. The earliest effect is probably due to the ability of these drugs to reduce the phagocytosis of antibody-sensitized cells by the RES, particularly the spleen.<sup>272</sup> In addition, there may be a rapid decrease in the concentration of cell-bound antibody, especially at high doses of steroids,<sup>282</sup> and this may further decrease the phagocytosis of red cells. Since the reduction of red cell antibody is accompanied by a rise in serum antibody,

the affinity of antigen for antibody has probably been altered by steroids.<sup>282</sup> Finally, in patients achieving remission, the concentration of serum antibody may fall to low levels<sup>282</sup> because of the known immunosuppressive properties of steroids.<sup>276</sup>

*Splenectomy* is recommended for patients whose anemia cannot be controlled by steroids during the acute phase of the disease, for those whose anemia requires continuous high-dose steroid therapy, or for those who have developed serious complications on relatively low doses of adrenocorticoids.<sup>219</sup> The results of splenectomy are improved greatly by selecting those patients who have demonstrated excessive sequestration of <sup>51</sup>Cr-labeled red cells within the spleen.<sup>213a,241</sup> Excessive sequestration is determined by withdrawing 25 to 50 ml venous blood and mixing this with ACD solution and 50 to 150 mc of <sup>51</sup>Cr. The mixture is incubated at room temperature for 30 to 60 minutes as in the procedure for measuring erythrocyte life span with <sup>51</sup>Cr-labeled red cells (page 197). Following re-injection of the tagged erythrocytes the anterolateral projection of the spleen is monitored with a unidirectional scintillation counter.<sup>259</sup> After equilibration over the spleen has been attained the anterior projections of the liver and the heart as well as of the spleen are counted. The relative uptakes of radioactivity over these organs are expressed in terms of splenic-precordial (S/P) and spleen-liver (S/L) ratios. Counting can be repeated daily or twice a week until a definite trend in the ratio becomes evident. A *splenic localization index (SLI)* has been devised as follows:

$$\frac{S/P}{S/P_0} \times 10 = \frac{d_{\max}}{d_{\max}} = SLI,$$

where S/P is the maximum change in the ratio,  $S/P_0$  is the initial ratio, and  $d_{\max}$  is the day on which the maximum ratio is found. Some workers have simply compared the ratio of radioactivity over the spleen with that of the liver.<sup>284</sup>

A maximum S/P ratio of 1.5 or more, an

S/L ratio of at least 2.0, and an SLI of 1.0 or higher indicate splenic sequestration and have been claimed to correlate well with other information regarding the major site of increased blood destruction. It is generally thought that patients manifesting marked splenic and little hepatic sequestration respond well to splenectomy.<sup>200,213a,211,292</sup> However, this view needs additional documentation since discrepancies do occur. In considering the desirability of splenectomy in AIHA subjects, it is therefore wise to give heed to clinical experience showing that patients with warm antibodies are more likely to respond than those with cold antibodies, that patients with splenomegaly respond better than those without a palpable spleen, and that those with incomplete antibodies, especially if present in relatively small amounts, are more likely to respond favorably to splenectomy than those with complete agglutinins or complement-fixing antibodies.

Before the introduction of steroid therapy it was reported that splenectomy was helpful in 50% of patients with AIHA,<sup>297</sup> but the duration of such remissions is not known. Even when steroids have been combined with splenectomy, high mortality rates have been cited.<sup>218</sup> Our own experience has not been as discouraging, although splenectomy should never be undertaken lightly; even in the best hands the mortality rate of the operation is considerable.<sup>217</sup> Atelectasis, subdiaphragmatic infections, and thromboembolic disease are the more common fatal complications. In addition, infants and children are particularly prone to develop pyogenic infections that carry high mortality rates (47%<sup>232</sup>), even when there is no other underlying disease.

Many cytotoxic drugs are known to be potentially immunosuppressive<sup>276</sup> and this property provides the theoretical basis for their use in AIHA. It is likely, however, that their therapeutic effect does not depend on immunosuppression alone, since clinical success need not be accompanied by a reduction in the titer of anti-red cell antibodies.<sup>248,287</sup>

Several drugs, including azathioprine,<sup>200,214,248</sup> 6-mercaptopurine,<sup>287</sup> thioguanine,<sup>287</sup> and cyclophosphamide,<sup>283</sup> have

enjoyed moderate success. Cyclophosphamide appears to be one of the most effective immunosuppressive agents available<sup>288</sup> and may have a better therapeutic index than do the other drugs.<sup>283</sup> None has been subjected to controlled trial.

As with steroid therapy, treatment is not without risk; serious infection may develop because general immunosuppression complicated by leukopenia and thrombocytopenia may occur. In addition, one needs to consider the increased incidence of tumors that accompanies long-term use of immunosuppressive agents,<sup>295,231</sup> as well as specific complications associated with some of these drugs, including bladder fibrosis with cyclophosphamide,<sup>252</sup> and their effect on the reproductive system.<sup>238,253,264,279</sup> In general, splenectomy is preferable to the use of cytotoxic drugs, but the latter may be useful if splenectomy fails and in patients unfit for a surgical procedure.

Although heparin has been useful in the management of some patients with AIHA,<sup>217,230,247,290</sup> its value as a practical measure of therapy remains uncertain.<sup>206,219</sup> The risk of hemorrhage and the difficulties of administration constitute serious disadvantages.

## Drug-Induced Immuno-hemolytic Anemias

Drug-induced immuno-hemolytic anemias are examples of diseases in which exposure to foreign antigens causes destruction of the sensitized individual's own cells. Although the number of recorded cases is relatively small, these anemias hold an unusual interest because they provide some insight into the nature of "autoimmune" phenomena in general. Furthermore, many cases of AIHA now considered to be "idiopathic" may eventually prove to be drug induced, especially when one considers that some drugs<sup>310</sup> seem to induce the production of antibodies against well-defined red cell antigens but have no recognizable cross reactivity with the trigger agent.

Drugs can lead to the destruction of red cells by several different immune mechanisms which have been named after the drug that best characterizes each group. These mechanisms are illustrated in Table 27-6. Currently recognized are those typified by (1) stibophen, (2) penicillin, and (3)  $\alpha$ -methyldopa; other drugs that act similarly are listed in Table 27-7.

### Drug-Induced Immuno-hemolytic Anemia of the Stibophen (or "Innocent Bystander") Type

#### Pathogenesis

Drug-induced hemolytic anemia of the "innocent bystander" type was first described by Harris (1954) in a patient being treated with stibophen because of schistosomiasis.<sup>330</sup> It was shown that the patient's serum contained a factor that could sensitize his own or foreign red cells to become Coombs' positive; that sensitization could lead to agglutination of normal cells and to complement-dependent lysis of trypsinized red cells or cells from a patient with paroxysmal nocturnal hemoglobinuria; and that all of these reactions, including the hemolytic reaction *in vivo*, depended on the presence of the drug.

It was subsequently shown that the deposition of anti-drug antibodies on cell surfaces involved two separate but interdependent reactions.<sup>353</sup> First, anti-drug antibodies react specifically with a stable complex of the drug and some soluble noncellular macromolecule

to produce a relatively large antigen-antibody aggregate. In the second reaction the antigen-antibody complex is thought to settle out on various cell surfaces, such as red cells, platelets, or other tissues. This step is immunologically nonspecific since it does not involve specific interactions between red cell antigens and the antigen-binding sites of the anti-drug antibodies. Nevertheless, for reasons that are not yet clear, the adsorption of antigen-antibody complexes to cells seems to proceed as avidly as the interaction between drug and anti-drug antibody in the immunologically specific first step.<sup>353</sup> The anti-drug antibodies may be IgG, IgM, or both and usually have the ability to bind complement, which is ultimately responsible for the cell lysis.

Intravascular destruction of red cells by complement-dependent mechanisms proceeds very rapidly (see page 892), and red cell sensitization by antigen-antibody-complement complexes may therefore escape detection if Coombs' test is carried out with anti-gamma globulin sera only. If Coombs' test is performed with anti-complement or "non-gamma globulin" sera, however, its reaction characteristically is positive in patients with the stibophen type of hemolytic anemia, and may remain so for up to two months after the initial insult. This is due to the persistence of sublytic amounts of complement on many red cells that, for unknown reasons, have shed their drug-antibody complexes. Attachment of the complement components appears to be virtually irreversible and, when present in sublytic quantities, complement

Table 27-6. Reactions in Three Types of Drug-Induced Hemolytic Anemias

Type of Reaction	Role of Drug or Drug Metabolite	Nature of Attachment of Antibody to RBC	Antiglobulin Reaction	Mechanism of Cell Destruction
Hapten (penicillin)	Cell bound hapten	To cell bound drug	Ig	Agglutination
Innocent bystander ("stibophen")	Antigen in circulating antigen-antibody complex	Adsorption as part of antigen-antibody-complement complex	C	Complement lysis
"Alpha-methyldopa"	Triggers formation of anti red cell antibody. No cross reactivity with drug	To Rh site on RBC membrane	Ig	Agglutination

**Table 27-7. Drug-Induced Immuno-hemolytic Anemias**


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1	<i>Stibophen ("innocent bystander") type</i>
	Stibophen <sup>330,353,354</sup>
	Quinidine <sup>329,352</sup>
	Quinine <sup>345,353</sup>
	p-Aminosalicylic acid <sup>343,352,359</sup>
	Phenacetin <sup>326,343,345,352</sup>
	Sulfonamides <sup>324,355</sup>
	Insecticides <sup>346</sup>
	Dipyron <sup>333</sup>
	Antihistine <sup>342</sup>
	Chlorpromazine <sup>339</sup>
	Pyrimidin <sup>359</sup>
	Isoniazid (INH) <sup>350</sup>
2	<i>Penicillin type</i>
	Penicillin <sup>335,338,344,359</sup>
3	<i>Alpha methyl-dopa type</i>
	Alpha methyl-dopa <sup>340,358,359</sup>
	Mefenamic acid <sup>351</sup>
4	<i>Miscellaneous</i>
	Cephalosporin derivatives <sup>328,344,359</sup>

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components may constitute the only evidence of antecedent immune complex activity. Similar reactions have been documented for a number of other drugs that are listed in Table 27-7.

It is not at all certain how drug-specific antibodies are generated. Since all of these drugs are of low molecular weight, they undoubtedly behave as true haptens and are therefore incapable of eliciting an immune response unless they are first bound firmly to a carrier protein that must itself be immunogenic. Autologous proteins would therefore only qualify as carriers if they were sufficiently distorted by haptenic conjugation or some other process to make them appear "foreign." The mechanisms by which this might occur are not clear.

### Clinical Manifestations

The dose of the offending drug is usually small and hemolysis only occurs in the presence of the medication. In most patients the picture is one of acute intravascular hemolysis with hemoglobinemia and hemoglobinuria. There is a high incidence of renal failure<sup>359</sup> and disseminated intravascular co-

agulation has been described.<sup>356</sup> The laboratory findings are those characteristic of intravascular hemolysis (page 728). Occasionally this is accompanied by leukopenia and/or thrombocytopenia. Spherocytosis may be evident on the blood smear. The serologic characteristics of the hemolytic antibodies have been described previously. Occasionally the antibodies may have agglutinating properties only, especially those with specificity for para-aminosalicylic acid.<sup>350</sup>

### Treatment

It is imperative that use of the offending drug be discontinued immediately. In some patients with a mild hemolytic process no additional treatment may be necessary. When hemolysis has been extensive and the hemoglobin level is low, blood transfusions are required; however, these may be of limited benefit since the transfused cells may be destroyed as rapidly as the patient's own erythrocytes. If drug-antibody complexes are still circulating in the patient's serum, some difficulty may also be encountered in cross-matching.

Since the hemolytic process is mainly of the intravascular variety, steroids are unlikely to be of benefit. Renal failure becomes a problem in about half the patients with intravascular hemolysis of this type.<sup>359</sup> Proper management of this complication is critical and has been discussed in detail in Chapter 11.

### Drug-Induced Hemolytic Anemia of the Penicillin Type

#### Pathogenesis

The mechanisms responsible for penicillin-induced hemolytic anemia differ significantly from those responsible for the stibophen type of hemolytic anemia. While the offending antibody in both instances has specificity for the drug and not some component of the erythrocyte membrane, anti-penicillin antibodies can only bind to red cells if the drug is first firmly coupled to the

erythrocyte membrane, probably by covalent bonds.<sup>337</sup> This is to be distinguished from the stibophen type of hemolytic anemia, in which circulating antigen-antibody complexes settle out on red cells and then lead to complement-dependent lysis of their innocent host (above). Some degradation products of benzylpenicillin are able to bond covalently to proteins<sup>335,336,337</sup>; of these the benzylpenicilloyl group appears to be the "major" haptenic determinant of benzylpenicillin hypersensitivity, accounting for at least 95% of all benzylpenicillin derivatives reacting irreversibly with protein.<sup>335</sup> In addition, several "minor" determinants are formed. Although called "minor" because they occur in comparatively smaller amounts, they nevertheless have a great deal of clinical significance.<sup>335</sup> Both major and minor determinants, separately or in combination, may be involved in red cell sensitization by penicillin.<sup>337,349</sup>

Sensitization to penicillin occurs only when relatively large doses, perhaps of the order of 20,000,000 units per day or more, are used.<sup>337,359</sup> It is, however, noteworthy that high-dose intravenous penicillin therapy, though necessary for coupling of penicillin to red cells, does not usually result in a high-titered anti-penicilloyl antibody response.<sup>338</sup> The latter depends on the administration of intramuscular penicillin, given either before or after intravenous therapy.<sup>337</sup> Thus, both high-dose penicillin therapy and antibodies of high titer or great affinity are needed to produce Coombs' positivity or hemolysis. The anti-penicillin antibodies are usually IgG, warm reactive, and noncomplement binding, but exceptional cases of hemolytic anemia due to complement-mediated mechanisms have been described.<sup>332</sup>

### Clinical Manifestations

As mentioned previously, patients with penicillin-induced hemolytic anemia have almost always received large doses of the drug, usually over a protracted period. The hemolytic process may be brisk with a rapidly falling hemoglobin level, reticulocytosis, and indirect hyperbilirubinemia, but without

clinical evidence of intravascular hemolysis. <sup>51</sup>Cr-tagged red cells are rapidly removed from the circulation and there is marked accumulation of radioactivity over the spleen.<sup>348</sup> Red cell destruction probably occurs mainly within the spleen. Spherocytosis is only rarely present<sup>359</sup> and the blood shows no other diagnostic features. At the time of hemolysis, all patients give a strongly positive reaction, of the anti-gamma globulin type, to the direct Coombs' test. If the sensitizing antibody is eluted it is found to have specificity for penicillin derivatives only and not for antigenic determinants of the red cell membrane. If penicillin therapy is stopped, the hemolysis ceases quickly and the reaction to the direct Coombs' test gradually becomes weaker over a period of days or weeks.

### Treatment

If the patient is actively hemolyzing, penicillin therapy should be discontinued immediately and structurally related congeners should not be substituted. Blood transfusions may become necessary; transfused cells should survive well if penicillin therapy has been discontinued and most of the drug has been excreted. Since red cell destruction in penicillin-induced immuno-hemolytic anemia is mainly extravascular and presumably splenic,<sup>348</sup> steroids should be of benefit, although protracted hemolysis has been described in the face of steroid therapy.<sup>319</sup>

### Drug-Induced Hemolytic Anemia of the Alpha-Methyldopa Type

Alpha-methyldopa is a widely used anti-hypertensive drug that is known to be a potent inhibitor of dopa decarboxylase, both *in vivo* and *in vitro*. In 1966 it was first reported that a high proportion of patients receiving this drug gave a positive reaction to the direct antiglobulin test and that a few of them developed an overt hemolytic anemia serologically identical with idiopathic autoimmune hemolytic anemia.<sup>358</sup> These observations were subsequently confirmed.

## Pathogenesis

Although the mechanism by which alpha-methyldopa leads to antierythrocyte antibody production is unknown, several observations are of extreme interest. The autoantibody is only formed after prolonged therapy and this time interval is not shortened with reexposure.<sup>358</sup> When use of the drug is stopped, the antibody titer declines and the reaction to the Coombs' test becomes negative in a few weeks or months, depending on the strength of the original reaction.<sup>358</sup> Only occasionally does the antiglobulin reaction remain positive for as long as two years.<sup>359</sup> The antibodies readily sensitize normal red cells in the absence of the drug, and the reaction cannot be inhibited by pre-incubation of antisera with alpha-methyldopa or several of its metabolites or congeners.<sup>310</sup> This suggests that there is no cross-reactivity between the drug and the red cell antigen with which the antibody interacts. It is of particular interest that the anti-red cell antibodies developing during alpha-methyldopa therapy have specificity for Rh antigens and that the pattern of specificity is similar to that described in idiopathic autoimmune hemolytic anemia. Thus, while some antibodies appear to react preferentially with well-defined Rh antigen such as hr(c) or hr''(e), others react with antigenic determinants common to all erythrocytes except those of the Rh-null type, which lack all antigenic determinants of the Rh complex.<sup>320</sup> Alpha-methyldopa-induced anti-red cell antibodies may therefore have specificity for some basic and commonly shared structural component of the Rh substance. The Coombs' reaction is positive with anti-gamma globulin sera and eluted antibodies have shown considerable structural heterogeneity,<sup>320</sup> suggesting a polyclonal pattern of immune responsiveness. In addition to anti-red cell antibodies, other autoantibodies are demonstrable in a large number of patients receiving alpha-methyldopa. These include antinuclear factors, rheumatoid factor, and antibodies against gastric mucosa.<sup>358</sup> Thus anti-red cell antibodies constitute only one of a number of non-cross-reacting autoimmune

responses induced by alpha-methyldopa, making it even more unlikely that the antibodies have specificity for some congener or degradation product of the drug and simply cross-react with normal tissue components.

There is no good explanation for the induction of autoantibodies by alpha-methyldopa therapy. The obvious hypotheses have been summarized by Worledge,<sup>358</sup> but none of these seems entirely satisfactory. The importance of elucidating the alpha-methyldopa mechanism cannot be underestimated, however, since it illustrates the induction of an autoimmune process by an extrinsic agent that does not itself appear to participate in the immune process that it has generated. It follows that extrinsic causative agents, and particularly drugs and viruses, must be suspected in all cases of so-called AIHA. Indeed so-called AIHA of the alpha-methyldopa type has already been described in association with another drug, mefenamic acid.<sup>331</sup>

## Clinical Manifestations

Alpha-methyldopa produces a positive reaction to Coombs' test in about 15% of all patients who have received the drug for a period of three months or more,<sup>359</sup> but most of these patients are not anemic and show no evidence of increased hemolysis. The incidence of hemolytic disease has ranged from none to even as high as 5% of all patients taking the drug; an overall incidence of 0.6% was noted by Worledge in a summary covering 14 separate studies and nearly 1400 patients.<sup>339</sup> In spite of this low incidence the total number of autoimmune hemolytic anemias due to alpha-methyldopa exceeds the total number of all other drug-induced immuno-hemolytic anemias described so far.

The onset of hemolysis has ranged from 18 weeks to four years after the start of therapy.<sup>359</sup> It is invariably insidious and the course of the disease is slowly progressive. No specific clinical features have been described. The symptoms are primarily those of anemia. The hematologic features are identical to those described for AIHA due to incomplete warm reactive autoantibodies

(above) with evidence of accelerated red cell production and mild to moderate spherocytosis, a proportionately variable increase in reticulocytes, and mild to moderate indirect hyperbilirubinemia. Reaction to the Coombs' test is positive with anti-gamma G sera only. Occasionally, reactions to tests for antinuclear antibodies and rheumatoid factors also may be positive.<sup>358</sup>

### Treatment

Most patients with hemolytic disease due to alpha-methyldopa require no therapy other than withdrawal of the offending drug.<sup>339</sup> Often recovery is surprisingly quick,<sup>327</sup> and in these patients the hemoglobin level may begin to rise as soon as use of the drug has been stopped. When hemolysis is more severe or does not readily come under control, steroid therapy is advisable and is usually effective.<sup>321,354</sup> Nevertheless, three deaths have been reported among 34 patients so treated.<sup>321,354</sup> This is an unusually high death rate from AIHA, undoubtedly attributable in part to the fact that alpha-methyldopa therapy is often given to patients who already are at high risk due to serious cardiovascular disease; the occurrence of hemolytic anemia in such patients should therefore never be treated lightly.

Blood transfusions may be necessary, as in patients with idiopathic autoimmune hemolytic anemia (above), and, as in patients with AIHA, difficulties in cross-matching may be encountered.

## Immunohemolytic Anemias Due to Cold Reactive Antibodies

Some antibodies react most efficiently with red cell antigens at temperatures below 32° C and are therefore called "cold reactive antibodies." Autoantibodies of this type may give rise to one of two clinical syndromes, depending on the functional properties of the anti-red cell antibodies involved (Table

27-5). The *cold agglutinin syndrome* is the more common of the two and accounts for about a third of all patients with autoimmune hemolytic anemias, whereas the syndrome of *paroxysmal cold hemoglobinuria* due to hemolysins is rare and is most characteristically found in association with congenital or acquired syphilis. The antibodies giving rise to these clinical syndromes belong to separate immunoglobulin classes, interact with different red cell antigens, and may give rise to distinct clinical manifestations. The two syndromes will therefore be discussed separately.

### Cold Hemagglutinin Disease

#### Etiology and Pathogenesis

Many theories have been proposed to explain the production of anti-red cell antibodies. These have been discussed in relation to immunohemolytic anemias due to warm reactive antibodies (page 910) and are equally applicable to the production of cold reactive antibodies. The latter occur most commonly in association with infectious or malignant diseases, but sometimes take place in the absence of known underlying causes. The infections that most commonly give rise to cold reactive antibodies are those due to *Mycoplasma pneumoniae*,<sup>323,414,415</sup> although a few cases have also been described in association with infectious mononucleosis.<sup>416,421</sup> Most cold reactive antibodies arising during these infections have specificity for the I-i antigens (Chapter 11) of red cells,<sup>414,416,439,443</sup> but a few antibodies that react equally well with all adult cells, cord blood cells, and adult i cells have also been described, suggesting that these have specificity for some other antigen (SP<sub>1</sub>) common to all human red cells.<sup>415,427</sup> Some observers have suggested that the anti-I specificity of cold agglutinins in *M. pneumoniae* infections is due to the sharing of I type antigenic determinants by *M. pneumoniae* and human red cells,<sup>253,396</sup> but this has been denied by others.<sup>233</sup> Additional support for the shared antigen theory comes from the observation that rabbits

immunized with *M. pneumoniae* also produce high titers of anti-I cold agglutinins.<sup>386,396</sup> Antibodies of similar specificity are produced by immunizing rabbits with *L. monocytogenes*<sup>395,396</sup> and streptococcus MG.<sup>396</sup>

Cold agglutinins found in patients with malignant disease may have either anti-I or anti-i specificity,<sup>321,385,425,450</sup> whereas sera of patients with the idiopathic cold hemagglutinin syndrome almost always have anti-I or, occasionally, anti-Sp<sub>1</sub> specificity.<sup>113,450</sup> These antibodies have no demonstrable cross-reactivity with *M. pneumoniae*.<sup>395</sup> Cold agglutinins found in some normal sera usually react with the I antigen.<sup>423</sup>

Almost all cold agglutinins are IgM, although rare cases of IgA cold agglutinins have been recorded.<sup>450</sup> IgM antibodies readily fix complement and can be shown to lyse red cells in vitro if the pH is adjusted to 6.5 to 7.0.<sup>397</sup> Dissociation of the 19S anti-I molecule into single 7S components results in loss of complement-fixing ability, without destroying the antibody's ability to cause agglutination.<sup>392</sup> Sometimes, and particularly in idiopathic cold agglutinin disease, the concentration of cold agglutinins may be so high as to give rise to abnormal peaks in electrophoretic patterns or to result in the formation of cryoprecipitates when the serum is immersed in ice.<sup>411</sup> In the idiopathic form of the syndrome the cold agglutinins appear to be of monoclonal origin, as judged by electrophoretic and structural studies.<sup>383,384,401,450</sup> They are considered to represent a special form of plasma cell dyscrasia<sup>443</sup> (Chapter 53). The post-infectious variants of the disease are polyclonal in origin, suggesting a reaction to an underlying infectious stimulus.<sup>414,443,450</sup>

Cold agglutinins usually do not react with red cells at temperatures of 32° C or higher. As the temperature is lowered towards 0° C the agglutinin titer quickly and progressively increases and complement fixation occurs; but while antibody binding and complement fixation are optimal at low temperatures, complement is most lytic at high temperatures (40° C). Thus hemolysis only occurs in the temperature range of 10 to 30° C where these two activities overlap, with maximal lysis at

about 22° C; at this temperature a sufficient amount of antibody remains fixed to the cell, and complement lytic activity, though not optimal, is of sufficient magnitude to result in hemolysis<sup>443</sup> (Fig. 27-4). Occasionally the base of the triangle may broaden to the right, indicating a higher thermal range of hemolytic activity and a more serious form of the disease.

Since normal serum contains an inactivator of membrane-bound C3 (C3bINA, see Chapter 7), hemolysis only occurs if complement fixation is sufficiently rapid to outdistance the effects of the inactivator.<sup>423,425</sup> This is ordinarily the case with acute cold stress. If hemolysis does not occur, antibody disengages itself from the cell at higher temperatures, while inactivated complement (C3) remains fixed. These inactivated complement components are responsible for the positive "non-gamma globulin" Coombs' reaction seen in cold agglutinin disease (page 924). Red cells that have once escaped hemolysis become unusually resistant to further complement lysis.<sup>391</sup>

While complement-mediated lysis accounts for most of the red cell destruction in the cold agglutinin syndrome,<sup>421</sup> other factors may play a role, including the striking

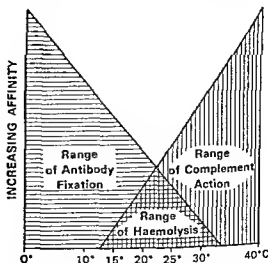


Fig 27-4. Schematic combination of the temperature ranges for cold agglutinin fixation and lytic complement action (From Schuboth, <sup>441</sup> courtesy of the author and *Seminars in Hematology*)



changes in red cell contour brought about by cold agglutinins. These are demonstrable by the electron microscope.<sup>440</sup> While the changes are reversible on warming, they impose considerable structural rigidity on cells trapped in cooled surface areas; indeed, agglutinated cells are known to be highly susceptible to mechanical trauma *in vitro*<sup>217</sup> and it is likely that a similar antibody-induced propensity to lysis exists *in vivo*.

### Clinical Manifestations

Chronic idiopathic cold agglutinin disease is predominantly a disease of the elderly, with a *clearcut peak incidence in the seventh and eighth decades of life*.<sup>217,443</sup> A few patients in their twenties and thirties are included in large series, however, and the disease is occasionally described in childhood.<sup>443</sup> The cold agglutinin syndrome seen in patients with malignant disease follows a similar age distribution, whereas that associated with viral pneumonia is most common during the third to fifth decades of life.<sup>217</sup>

The clinical manifestations are predominantly due to vascular disturbances or hemolysis and reflect the functional properties of the patient's cold agglutinins.<sup>441</sup> Thus patients with a high hemagglutinin and a high hemolysin titers suffer from vascular disturbances as well as hemolysis. A smaller group of patients have a high agglutinin titer but hemolytic activity may only be demonstrable by the use of trypsinized red cells, and such patients have vascular disturbances in the cold but no hemolytic disease. In a very occasional patient, intravascular hemolysis constitutes the predominant clinical feature and in such an individual a relatively low cold agglutinin titer is associated with unusually intense hemolytic activity *in vitro*.

The circulatory changes are commonly described as "*acrocyanosis*" and are caused by the intracapillary agglutination of red cells in those areas of the body that are cooled to temperatures encompassed by the thermal range of the antibody in question. This results in a striking discoloration of the skin that may vary from a sick-looking white to deep blue-violet. The color changes may be

accompanied by numbness and occasionally by pain. Although the vascular disturbances are potentially reversible, signs of chronic tissue damage, including trophic changes of the extremities and even gangrene, may eventually supervene.<sup>397,400,430,433,445</sup> Acrocyanosis or "Raynaud's phenomenon" may involve any part of the body, but the exposed distal extremities, the tip of the nose, and the ear lobes are most commonly affected. Acrocyanosis may be precipitated by a useful diagnostic maneuver, the *ice cube test*, which produces a circumscribed area of intravascular agglutination and acrocyanosis in the hyperemic palm.<sup>443</sup> While acrocyanosis may be the result of red cell agglutination, tissue gangrene is often associated with the presence of cryoglobulins.<sup>397,400,433</sup> These may produce a localized form of the hyperviscosity syndrome (page 1625), thereby contributing to the production of acrocyanosis and irreversible tissue damage.

Patients suffering from the idiopathic cold agglutinin syndrome usually develop a *chronic hemolytic process* of moderate severity; it is rare for the hemoglobin concentration to drop below 7 g/dl.<sup>394</sup> In northern climates the anemia appears to be worse during the winter,<sup>217</sup> but patients with high titers of cold agglutinins usually have a chronic hemolytic process regardless of temperature. When an acute cold-induced episode of intravascular hemolysis and hemoglobinuria is superimposed on the chronic disease, it often is accompanied by fever, chills, or renal shutdown.

Hemolytic disease due to cold agglutinins rarely accompanies pulmonary infections due to *M. pneumoniae*,<sup>444</sup> but when it does the hemolysis is of more abrupt onset and may occasionally be severe. The process is self-limited, however; the cold agglutinin titer usually drops to normal levels within three to four weeks. Other secondary forms of the cold agglutinin syndrome, usually accompanying malignant disease of lymphoid origin, tend to be more chronic. Hemolytic disease is not usually a prominent feature of the clinical picture, which is most frequently dominated by the underlying illness.<sup>443</sup>

In the idiopathic variant there are no strik-

ing *physical findings* other than those related to acrocyanosis, anemia, and perhaps mild jaundice. The patient usually is in good general condition, the spleen is frequently just palpable at the costal margin, and the liver may be slightly enlarged. Patients with cold hemagglutinin disease associated with infection or a malignant condition will have signs of the underlying disorder.

The effect of cold on intravascular hemolysis may be demonstrated by the "Ehrlich finger test." After the venous return of a finger has been occluded with a rubber band, the digit is immersed in cold water (20° C) for 15 minutes. At the same time, to serve as a control, another digit is occluded but is immersed in warm water (37° C). The centrifuged capillary blood obtained from the finger that was immersed in cold water is usually markedly hemolyzed as compared to that of the control.

## Laboratory Findings

### Blood

The hemoglobin and hematocrit values reflect the degree of hemolysis and may show considerable variation in relation to seasonal and other factors. The reticulocyte count is correspondingly elevated. Red cell counts and blood smear preparations may be made with difficulty unless they are carried out on fresh, warm specimens and the equipment is prewarmed to prevent cold agglutination. This is particularly true when dealing with blood containing high titers of antibody, as an increased thermal range is usually found under these conditions. Often cold agglutination is first discovered in the counting chamber, and may be confirmed by demonstrating the dissolution of clumps upon warming. Aside from some degree of autoagglutination, the blood smear is not remarkable and simply reflects the regenerative effort of the marrow. Spherocytosis is usually not striking and nucleated red cells are seldom present in large numbers.

The serum bilirubin is rarely more than

3 mg/dl and is predominantly indirect reacting. There may be other signs of mild intravascular hemolysis such as hemoglobinemia, low or absent haptoglobin levels, and chronic low-grade hemoglobinuria and hemosiderinuria. The latter may become more conspicuous in cold weather.

The stool contains an increased amount of stercobilinogen.

### Serologic Findings

In the presence of significant hemolysis the cold agglutinin titer (assessed at 4° C) may range from  $\frac{1}{1000}$  to  $\frac{1}{1,000,000}$ , but titers of  $\frac{1}{20,000}$  are more usual. In patients with infections the titer generally is lower ( $< \frac{1}{4000}$ ) even in the presence of hemolysis.<sup>414</sup> As was pointed out above, the hemolytic activity of the serum correlates more closely with the hemolysin titer than with the hemagglutinin titer. When measuring agglutinin titers it must be remembered that the titer may rise 10,000- to 100,000-fold when the temperature is lowered from 30° C to 0° C, and that the process is readily reversible if the test system is allowed to rewarm. In addition, the titer is usually higher when normal red cells are used instead of the patient's own erythrocytes. This may be related to the prior binding of complement components to the patient's red cells.<sup>350,393,394</sup> Most cold agglutinins have anti-I specificity, but anti-i and anti-Sp<sub>1</sub> antibodies also occur. The structural properties of cold agglutinins were discussed earlier. Complement levels are usually decreased while hemolysis is in progress.<sup>350</sup>

The reaction to the direct Coombs' test is positive even if the erythrocytes are not cooled prior to testing. It is specific for complement components; anti-gamma globulin sera typically give no reactions. The previously described "incomplete cold antibodies" are artifacts occasioned by the use of nonspecific Coombs' sera; agglutination was, in fact, caused by the reaction of anti-complement antibodies contained in such sera with complement components fixed to the patient's red cells.<sup>217</sup>

## Therapy

### Primary Chronic Cold Agglutinin Disease

Many patients have a mild, chronic hemolytic process requiring no specific therapy. When exacerbations of moderate severity occur they often respond to simple measures such as warmth and bed rest. It is advisable to avoid giving *blood transfusions* since they may be associated with acceleration of the hemolytic process. Because this reaction has been attributed to the infusion of fresh complement components, the use of washed red cells has been recommended. However, it seems likely that the patient's own erythrocytes are protected by the prior binding of sublytic amounts of complement.<sup>390,393,394</sup> According to this theory the donor erythrocytes should be responsible for the accelerated hemolysis and this has indeed been documented.<sup>395</sup> There may also be problems in cross-matching, especially if the procedure is carried out at temperatures below 32° C.

*Splenectomy* and *steroids* are usually not effective in the therapy of chronic cold agglutinin disease<sup>443</sup> although occasionally success has been reported. However, such success probably is attributable to other concomitant care; bed rest is usually as effective as therapy with ACTH or cortisone.<sup>358</sup> The lack of success with splenectomy or steroids is not surprising when one considers the pathogenesis of cold hemolysis.

The most rational therapy of the cold agglutinin syndrome is aimed at the suppression of antibody production. This can usually be accomplished by the use of agents such as cyclophosphamide<sup>443</sup> or chlorambucil.<sup>219, 412,431</sup> Drastic reductions in titer and amelioration of the hemolytic process have been reported.

### Secondary Cold Agglutinin Disease

The treatment of *acute transient cold agglutinin disease accompanying viral pneumonia* presents fewer problems than does the primary chronic disease, since the disease is

self-limited and cold agglutinin titers usually return to harmless levels within two to three weeks. Usually no specific therapy is necessary. When the cold agglutinin syndrome accompanies *malignant disease*, therapy must be aimed at the underlying disorder.

## Paroxysmal Cold Hemoglobinuria

Paroxysmal cold hemoglobinuria (PCH) is characterized by the sudden passage of hemoglobin in the urine following local or general exposure to cold. Since this is such a dramatic occurrence, PCH was the first of all hemolytic anemias to be recognized and described.<sup>217</sup> The clinical features were well defined by the end of the 19th century, including the effect of cold exposure,<sup>390,405,406</sup> the derivation of the urinary pigment from circulating red cells,<sup>392,419,434</sup> and the etiologic relationship to syphilis.<sup>401</sup> In 1904, Donath and Landsteiner<sup>391</sup> published their classical paper outlining the basic pathogenetic mechanisms of the disease. They showed that the hemolysis was most likely due to an "autolysin" that unites with the patient's erythrocytes at low temperatures, and that labile serum factors cause lysis of the sensitized cells if the temperature is subsequently raised.

## Etiology and Pathogenesis

The Donath and Landsteiner (D-L) antibody has since been identified as a 7S IgG antibody that is a remarkably powerful hemolysin, even in relatively low concentrations. It has frequently been referred to as "biphasic" because chilling to 0° C, followed by warming to body temperature, as in the classic D-L test, leads to the most efficient lysis. The requirement for prior cooling depends on the fact that D-L antibodies bind to red cells most avidly at temperatures of less than 15° C, which are too low for lysis by complement. Nevertheless, antibodies of greater thermal range are sometimes encountered; these will lead to "monophasic" lysis if the cell-serum suspension is simply allowed to stand at 15 to 25° C.

Maximum hemolysis is only seen if complement also is present at the time of antibody binding in the cold phase, perhaps because complement facilitates the fixation of antibody to the cell<sup>217</sup> or because it retards the rate of its elution on subsequent rewarming.<sup>410</sup> Hinz and coworkers showed that D-L antibodies from several patients caused no more than a trace of lysis if complement had been omitted in the cold phase.<sup>408,410</sup> The complement component required during the cold phase has been identified as IIS Clq<sup>409</sup> (Chapter 7). This fixes to the cell surface in the presence of antibody and is followed by fixation of the C1r and C1s components in the presence of calcium ions; during the warm phase C4, C2, magnesium, and C3,5,6,7,8 and 9 in sequence are necessary for lysis.<sup>410,411</sup>

Since the D-L antibody is an IgG immunoglobulin, red cells sensitized with it will give a positive reaction to Coombs' test with an anti- $\gamma$ G serum, if the test is carried out in the cold. If sensitized cells are first washed at 37° C, the anti-gamma globulin serum will not bring about agglutination, although a broad-spectrum serum or an anti-complement Coombs' serum will agglutinate these cells at any temperature, provided the original sensitization to the D-L antibody occurred in the presence of complement.

Although the D-L antibody usually is thought of as a hemolysin, it is likely that the antibody regularly causes some agglutination as well as lysis, especially if the former is looked for in the absence of complement.<sup>217,418,422</sup> Unlike cold agglutinins which have anti-Ii specificity, cold hemolysins of the D-L type have specificity for the Pp blood group system<sup>422</sup> (Chapter 11). Although the antibody specificity initially seemed to be identical to that of the anti-Tja isoantibody found in the serum of pp subjects, more recent work has established that p<sup>k</sup> cells as well as pp cells are unaffected by the D-L antibody. Its specificity is therefore more correctly referred to as anti-P.<sup>421</sup> The specificity of the antibody seems to be the same in syphilitic and non-syphilitic patients.

The antibody is classically described in patients with tertiary syphilis or congenital

syphilis, but it is also seen in the absence of either disease. Thus of eleven patients studied by Worledge and Rousso<sup>421</sup> only three were definitely syphilitic and five were definitely non-syphilitic. Of the latter group, the manifestations in one followed measles and in another it followed mumps infection. Other investigators have also demonstrated the presence of the D-L antibody for short periods following viral infections, especially measles and mumps.<sup>217,381,411,445</sup>

The reason for the development of the D-L hemolysin in any of these situations is not known. Although a third of all patients with this antibody have a positive Wassermann reaction, the D-L hemolysin has been shown to be distinct from the antibodies that give rise to the positive reactions to the various biologic tests for syphilis.<sup>408</sup> It may be present transiently or for prolonged periods and does not always disappear with antiluetic therapy. Since PCH is rare but has been described in more than one family member,<sup>217</sup> it is likely that genetic factors controlling immune responsiveness<sup>260</sup> play a role in determining who develops the D-L autoantibody.

### Clinical Manifestations

The most striking manifestation of PCH is the passage of dark-brown or black urine after local or general exposure to cold. In some patients the disease is precipitated by brief exposure to moderate cold, whereas, in others, prolonged exposure to severely chilling temperatures is required. Systemic symptoms appear any time from a few minutes to eight hours after chilling and may consist of aching and pain in the back, legs, or abdomen; cramps; general malaise; headache; vomiting; diarrhea; and, finally, severe shaking chills and fever. The hemoglobin-containing urine is passed during the attack or shortly thereafter, but gross discoloration may only be evident in the first two or three specimens. Following the acute attack the patient is weak, pale, and may be slightly icteric. The spleen often is somewhat enlarged, and the liver may be palpable. Vasomotor disturbances such as urticarial

wheals, unusual sensitivity to frostbite, and acrocyanosis have been described.<sup>217</sup>

Sometimes the symptoms are milder; hemoglobinemia may occur without hemoglobinuria and only moderate fever and icterus may develop. Recovery from the attack usually is rapid and between attacks the patient may be entirely symptom free.

## Laboratory Findings

### Blood

Hematologic findings are those typical of acute intravascular hemolysis (page 728) and the severity of the changes reflects the severity of the attack. During sudden massive hemolytic episodes the hemoglobin level may drop rapidly, the plasma becomes markedly red, and, once the haptoglobin has been saturated, hemoglobinuria occurs. At this time, methalbumin also becomes detectable in the plasma and a mild to moderate elevation of unconjugated bilirubin follows. No diagnostic changes in the morphologic appearance of the red cells are seen, but erythrophagocytosis is a common feature of acute attacks. Leukopenia develops early and this is later followed by granulocytic leukocytosis; a considerable number of immature leukocytes may be present in the blood smear. In persons subjected to continuous cold weather and repeated attacks, chronic hemolytic anemia with hemosiderinuria may develop.

### Urine

The urine contains hemoglobin and methemoglobin, giving it a dark-red, brown, or almost black appearance. Occasionally red cell casts or ghosts are found in the urinary sediment, and albuminuria has been described. As already stated, the urinary abnormality may be confined to the first two or three specimens obtained after an attack.

### Serologic Findings

The reaction to the direct Coombs' test is positive during the attack and shortly thereafter if a complement-specific ("non-gamma

globulin") antiserum is used. The positive reaction is due to those cells that are coated by inactivated complement components or sublytic quantities of the active forms. Antibodies can usually not be demonstrated on red cells, since these dissociate on warming. The reaction to the indirect Coombs' test may, however, be positive if the test is performed in the cold, and, as pointed out earlier, normal erythrocytes give better reactions than do the patient's own cells.

In addition to the Ehrlich finger test, described earlier (page 924), a number of other tests are available for demonstrating antibodies in vivo and in vitro.

In the *Rosenbach test*, an attack of hemoglobinemia and hemoglobinuria may be produced by immersing the patient's hands or feet in ice water for 10 to 20 minutes. Occasionally, all four extremities must be immersed in order to produce a positive reaction. Since serious symptoms may arise in sensitive patients this test is not recommended for general use and should certainly not be attempted unless it is known that less exposure, as in the Ehrlich test, will not produce hemoglobinemia.

In the *Donath-Landsteiner test*, hemolysis is demonstrated following chilling of the blood in vitro. A rough test (MacKenzie)<sup>124</sup> is carried out as follows: Two or 3 ml of blood are taken from the patient and the same amount from a normal individual. Each sample is placed in a separate test tube and allowed to clot. Both tubes are then immersed in ice water for 10 minutes and subsequently warmed in a water bath for 30 minutes at 37° C. In a positive response, hemolysis is found in the blood taken from the patient, whereas the serum in the control tube is clear.

A more satisfactory test (modified from MacKenzie) is performed as follows:

All syringes, tubes, and salt solutions used in carrying out the test are warmed. About 10 ml blood are taken from the arm vein of the patient. Half of this blood is placed in a dry, clean centrifuge tube, allowed to clot, and centrifuged; the serum is then removed. The remainder is placed in a bottle containing anticoagulant and some of these red cells are washed three times in warm 0.85% so-

drum chloride solution. Then a 5% suspension of cells is made. About 2 ml of the suspension will be required.

Blood is obtained from a normal individual of the same blood group as the patient and is prepared in the same way that the patient's blood is prepared. Complement is prepared by making a 1:10 dilution of fresh guinea pig serum.

Six tubes are placed in a rack and 0.2 ml of complement is measured into each; 0.1 ml of saline solution is pipetted into tubes 1, 2, 3, and 4 and 0.6 ml is pipetted into tubes 5 and 6; 0.5 ml of the patient's serum is measured into tubes 1 and 3, and an equal volume of control serum is measured into tubes 2, 3, and 4. Then 0.2 ml of the suspension of patient's red cells is placed in tubes 1, 4, and 5 and the control red cells are placed in tubes 2, 3, and 6. The set of six tubes is then immersed in ice water for 10 to 30 minutes, after which it is warmed in a 37° C water bath for 30 minutes. When the reaction for cold hemolysis is positive, hemolysis will be found in tubes 1 and 3, whereas the other tubes will be clear.

## Treatment

Treatment for syphilis is given when this disease is present; clinical improvement of the PCH appears to occur in the majority of such patients.<sup>217</sup> Failures have been reported, however, and in some patients the reaction to the D-L test has remained positive even though the hemolytic disease has been cured.<sup>217</sup> No specific therapy is available for patients suffering from non-syphilitic forms of PCH, but in those with PCH accompanying acute viral infections the disorder is usually of short duration and no therapy is required. Avoidance of cold and damp is the only effective measure for individuals suffering from chronic idiopathic PCH. There seems to be no good indication for the use of steroids in the treatment of any of these patients. Immunosuppressive drugs, such as cyclophosphamide, have, to our knowledge, not been tried, although they would constitute a logical choice in patients suffering from chronic PCH of unknown cause.

Antihistamines and steroids are useful in the control of urticarial lesions.

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## The Red Cell Fragmentation Syndromes

### Red Cell Fragmentation Due to Cardiac Abnormalities

#### Red Cell Fragmentation in Association with Small Vessel Disease (Microangiopathic Hemolytic Anemia)

##### Experimental Models of Pathogenesis The Hemolytic Uremic Syndrome

##### Thrombotic Thrombocytopenic Purpura Disseminated Intravascular Coagulation Microangiopathy Associated with Immune Mechanisms

##### Giant Hemangiomas and Hemangioendotheliomas

##### Disseminated Carcinoma Eclampsia and Pre-eclampsia Malignant Hypertension March Hemoglobinuria

crescents, helmets, triangles, and/or microspherocytes (Fig. 28-1). These morphologic features readily differentiate the red cell fragmentation syndromes from other acquired hemolytic anemias.

Hemolytic anemias due to red cell fragmentation have been associated with abnormalities of the heart and great vessels, or with disease of small vessels (Table 28-1). These two groups differ in regard to etiology, pathogenesis, and prognosis, and require different approaches to therapy. They will therefore be discussed separately.

### Red Cell Fragmentation Due to Cardiac Abnormalities

#### Etiology

Soon after the advent of open heart surgery it became apparent that the postoperative course of some patients was complicated by the development of anemia of varying severity. The earliest observations were made in recipients of Hufnagel ball valves that had been inserted into the thoracic aorta.<sup>39,40</sup> Experiments with Hufnagel valves in dogs subsequently established intravascular hemolysis as the cause of the anemia.<sup>45</sup> Unfortunately, none of these workers studied the morphologic appearance of the erythrocytes in detail and the discovery of fragmented red cells as

WHEN red cells are subjected to excessive physical trauma within the cardiovascular system they may undergo premature fragmentation and lysis. Sometimes the insult is sufficiently severe to cause significant intravascular hemolysis with hemoglobinemia, hemoglobinuria, hemosiderinuria, and shortened red cell survival. The hallmark of this type of hemolysis is the fragmented red cell or *schistocyte* (page 543). This is a cell that has successfully resealed its membrane after losing part of its envelope and cytoplasm, and continues to circulate for a short time before being removed by the reticuloendothelial system. Schistocytes may take the form of

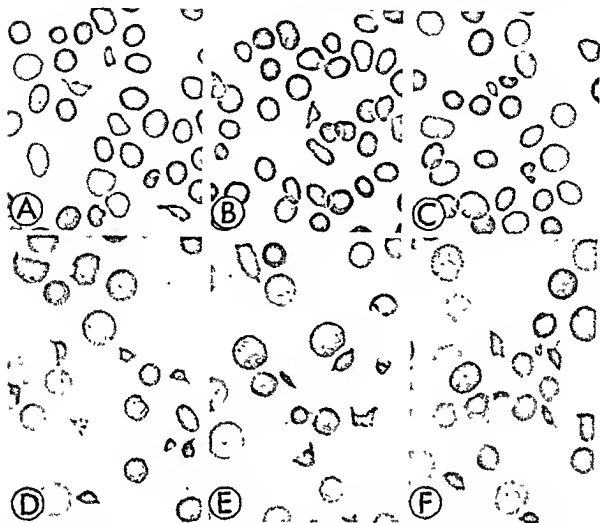


Fig. 28-1. Schisocytes in red cell fragmentation syndromes. A, B, and C are from patients with artificial heart valves. D, E, and F are from patients with microangiopathic anemia.

a characteristic feature of this type of anemia was not made until 1961, when Sayed, Dacie, and their coworkers reported these morphologic alterations in a patient who had developed severe and persistent intravascular hemolysis after repair of an ostium primum defect with Teflon.<sup>41</sup> Since then many reports have described a variety of patients in whom fragmented erythrocytes or clinically detectable intravascular hemolysis was associated with a wide variety of structural defects of the heart or great vessels (Table 28-1).

Surgically inserted prosthetic devices have furnished the most striking examples of red cell fragmentation. These include valves of the ball-and-basket variety,<sup>4,19,27,32,37,38</sup>

disk-type prostheses,<sup>52</sup> Teflon leaflets,<sup>51,57</sup> and synthetic valves made of Dacron cloth impregnated with rubber,<sup>12</sup> as well as valve homografts<sup>39</sup> and autograft valvoplasties.<sup>11,46</sup> Most of the valve prostheses associated with hemolytic disease have been of the aortic variety, but occasional cases of hemolysis due to mitral valve replacement<sup>26,52</sup> and unsuccessful mitral valvoplasty<sup>53</sup> also have been reported.

Intracardiac patch repairs of various types also can lead to intravascular hemolysis<sup>41,43,50</sup>; most reported cases have occurred in patients with Teflon patches used in the repair of ostium primum defects. In addition, intravascular hemolysis has been noted in a

**Table 28-1. Causes of Red Cell Fragmentation**

- A In association with abnormalities of the heart and great vessels**
- 1 Synthetic valvular prostheses<sup>4 17 19 27 32 37,38, 51 52 57</sup>
  - 2 Valve homografts<sup>38</sup>
  - 3 (Autograft) valvoplasties<sup>11 44, 58</sup>
  - 4 Intracardiac patch repairs<sup>41 43 50</sup>
  - 5 Unoperated valve disease<sup>3 7 9 14 20 49 55</sup>
  - 6 Coarctation of the aorta<sup>34, 54</sup>
- B In association with small vessel disease—microangiopathic hemolytic anemia**
- 1 Hemolytic uremic syndrome<sup>22 91 142</sup>
  - 2 Thrombotic thrombocytopenic purpura<sup>152 173</sup>
  - 3 Disseminated intravascular coagulation
    - (a) With sepsis<sup>234 239</sup>
    - (b) Purpura fulminans<sup>218</sup>
    - (c) Heat stroke<sup>245</sup>
    - (d) Abruptio placentae<sup>47 201</sup>
  - 4 Microangiopathy associated with immune mechanisms
    - (a) Lupus erythematosus<sup>75</sup>
    - (b) Acute glomerulonephritis<sup>73, 99</sup>
    - (c) Homograft rejection<sup>225</sup>
    - (d) Micropolyarteritis nodosa<sup>75, 149</sup>
    - (e) Scleroderma<sup>240</sup>
    - (f) Wegener's granulomatosis<sup>207</sup>
    - (g) Systemic amyloidosis<sup>222</sup>
  - 5 Hemangiomas
    - (a) Giant hemangioma<sup>220 237</sup>
    - (b) Hemangioendothelioma of the liver<sup>195</sup>
    - (c) Plexiform lesions in pulmonary hypertension<sup>247</sup>
  - 6 Disseminated carcinoma<sup>75 202 209 214 222 227 241 246</sup>
  - 7 Pregnancy
    - (a) Eclampsia and preeclampsia<sup>200 212 236</sup>
    - (b) Postpartum hemolytic uremic syndrome<sup>204, 211</sup>
  - 8 Oral contraceptives<sup>203</sup>
  - 9 Malignant hypertension<sup>75, 197 205, 226 241</sup>

aortic valve disease, especially aortic stenosis, but occasionally red cell fragmentation has been observed in patients with mitral valve involvement. Intravascular hemolysis also has been reported in a patient who had a ruptured aneurysm of the sinus of Valsalva,<sup>16</sup> in one with coarctation of the aorta,<sup>36</sup> and in another with coarctation and a bicuspid aortic valve.<sup>51</sup> When hemolytic disease accompanies valvular heart disease for which surgical treatment has not been given, it is rarely severe and in most affected patients the hemolysis is minor and clinically unimportant.

### Incidence

The exact incidence of intravascular hemolysis due to intracardiac prostheses is difficult to determine, since it undoubtedly depends on the severity of the defect, the expertise of the surgeon, and the sensitivity of the methods being used to study patients for the presence of hemolysis. Clinically significant hemolytic disease has been reported in 5 to 25% of patients with various types of valvular prostheses,<sup>24, 27</sup> usually replacing defective aortic valves, and in about 5% of patients with Teflon repairs of ostium primum defects.<sup>209</sup> The incidence of clinically detectable hemolysis in patients with mitral valve prostheses is considerably lower. When sensitive techniques such as red cell survival,<sup>3, 20 56</sup> haptoglobin levels,<sup>1, 3 7, 49</sup> or lactate dehydrogenase isoenzyme levels<sup>1, 23</sup> have been used, however, it has been found that a majority of patients with aortic valvular prostheses give evidence of mild intravascular hemolysis.<sup>1, 3, 7, 20, 56</sup>

The incidence of intravascular hemolysis in selected patients with aortic valve disease who have not been operated upon has ranged from about 5%<sup>28</sup> to 67%<sup>5</sup> as studied by <sup>51</sup>Cr survival techniques,<sup>5, 20, 28</sup> haptoglobin levels,<sup>7, 49</sup> and lactate dehydrogenase isoenzymes.<sup>23</sup> Undoubtedly, the severity of the valvular disease is an important variable.

### Pathogenesis

Red cell survival studies have clearly shown that the red cell fragmentation associ-

patient with tetralogy of Fallot who had a Dacron tube inserted between the aorta and the pulmonary artery.<sup>48</sup> When the artificial channel became obliterated by thrombus, the hemolytic process stopped.

Although red cell fragmentation is most strikingly associated with intracardiac surgical procedures, intravascular hemolysis due to similar mechanisms also has been described in many patients with valvular heart disease who have not been treated surgically.<sup>5, 8, 14, 20, 49, 55</sup> Most commonly this has been noted in patients suffering from severe

ated with intracardiac abnormalities is due to an extracorporeal defect.<sup>19,27,41,43,44</sup> Several mechanisms have been postulated to account for the intravascular nature of the hemolysis and the appearance of the characteristic fragmented cells.

*Direct mechanical trauma*, for instance by the closure of prosthetic valves, has been postulated; it is known to occur in other conditions such as march hemoglobinuria,<sup>253</sup> but since many patients with prosthetic valves do not have clinically significant hemolysis, it is unlikely that valve closure itself is responsible for much mechanical disruption of red cells. It is also unlikely that the presence of prosthetic materials per se contributes to red cell fragmentation,<sup>27,30,45</sup> although bare Teflon has been found at reoperation in some patients.<sup>41,50</sup> It would seem more likely that both the hemolysis and the lack of endothelialization depend on the presence of a third factor, such as turbulence, as discussed below.

Since the hemolysis of microangiopathic hemolytic anemia is related to the deposition of fibrin in small blood vessels (page 938) it has been postulated that similar mechanisms may be operative in the hemolytic anemia associated with cardiac defects. Fibrin deposition within the heart is not a common finding at autopsy, however<sup>27</sup>; in addition, only very fine fibrin strands are capable of fragmenting red cells (page 939), and it is unlikely that such thin fibrin threads could survive, for example, in the turbulence of the left ventricular outflow tract.

A positive reaction to the direct Coombs' test has been observed in a few patients with prosthetic valves<sup>22,33,34</sup> and occasionally in patients with severe aortic valve disease in the absence of surgical intervention.<sup>55</sup> It has been suggested that mechanical damage may have exposed subsurface antigens which then elicited the production of autoantibodies; or transfused lymphocytes may have established a mild graft-versus-host syndrome.<sup>34</sup> However, when the antibody is eluted it behaves as a panagglutinin, reacting with all normal cells tested. Thus it is unlikely that the antibody is specific for hidden determinants or that it is the result of graft-versus-host dis-

ease. Neither is the foreign material used during surgical procedures responsible for the development of autoantibodies.<sup>34</sup>

The most common feature in recorded cases of hemolysis following the insertion of prosthetic devices has been the existence of some form of hemodynamic defect, such as regurgitation through or around aortic prostheses,<sup>27</sup> mitral insufficiency in patients whose AV canal defects had been repaired by Teflon patches,<sup>43</sup> and regurgitation of blood through a Dacron tube inserted between the aorta and the pulmonary artery in a patient with tetralogy of Fallot.<sup>48</sup> Extreme turbulence is a common factor in all of these patients and it has been suggested<sup>20</sup> that this is responsible for hemolysis, rather than the direct buffeting of red cells against unphysiologic obstacles. It has been shown that a shearing stress in excess of 3000 dynes/cm<sup>2</sup> can easily cause mechanical hemolysis in vitro,<sup>31</sup> and that turbulence resulting in this degree of stress may readily develop in the presence of regurgitant defects between the aorta and the left ventricle in situations where the lumen of the aortic prosthesis is small relative to the stroke volume, or when the ball of a ball valve is large relative to the diameter of the aorta. Regurgitant jets through clefts in the mitral valve may establish similar areas of turbulence.

### Clinical Manifestations

There are no distinctive clinical features, with the exception of those related to pre-existent heart disease or cardiac surgery. Sometimes the development of hemolysis coincides with severe deterioration of cardiac function because of the tear of a valve cusp, or the loosening of valve attachments. When the hemolysis is clinically significant, jaundice frequently is obvious, but hemoglobinuria may not be detectable by the naked eye.

### Laboratory Findings

The blood picture varies widely, depending on the severity of the hemolytic process. The hemoglobin level may be normal if the hemolysis is compensated; or it may be ex-

tremely low. Most cells are normocytic and normochromic, although macrocytosis and polychromatophilia will be present in association with a brisk reticulocytosis. When hemolysis and hemosiderinuria have been prolonged, hypochromia due to iron deficiency may develop. The characteristic red cells, however, are the *fragmented erythrocytes* or *schistocytes* (Fig. 28-1), which are identical to those present in patients with microangiopathic hemolytic anemia (see below). The number of fragmented cells seen in the blood smear directly reflects the severity of the hemolytic process and in smears from patients with mild cases none may be apparent.<sup>13</sup> The *bone marrow* shows increased erythropoietic activity and, if the hemolysis is of long standing, the marrow iron stores may be depleted due to hemosiderinuria.

The serum *bilirubin* is slightly or moderately raised and the plasma hemoglobin level may be elevated. The haptoglobins are depleted and methemalbumin is found in the plasma. Lactate dehydrogenase levels, and especially those of isoenzyme LDH 1, are probably always elevated, at least in patients with prosthetic devices.<sup>13, 23</sup>

Hemoglobinuria and hemosiderinuria are found in patients with the more severe cases and may be constant. Hemosiderinuria is present in many patients when hemoglobinuria is not detectable.

The reaction to Coombs' test usually is negative, but occasionally the reactions are positive (see above).

## Treatment

When it is necessary and feasible the cardiac defect should be repaired; only occasionally does the condition causing the hemolysis improve spontaneously.<sup>11</sup> Since the severity of the hemolytic process is increased by physical activity,<sup>42</sup> bed rest is mandatory during acute exacerbations. When red cell fragmentation is accompanied by a positive reaction to Coombs' test, steroids may reduce the degree of hemolysis.<sup>34</sup> When iron deficiency has developed because of prolonged hemosiderinuria, iron therapy is of distinct benefit.<sup>37, 53</sup>

## Red Cell Fragmentation in Association with Small Vessel Disease (Microangiopathic Hemolytic Anemia)

In 1952, Symmers<sup>134</sup> introduced the term "microangiopathic hemolytic anemia" to describe a clinical syndrome now commonly referred to as "thrombotic thrombocytopenic purpura" (see below). The term was subsequently popularized by Brain, Dacie, and Hourihane<sup>75</sup> and is now used to designate any hemolytic anemia that is due to red cell fragmentation occurring in association with small vessel disease (Table 28-1).

### Experimental Models of Pathogenesis

In man, microangiopathic hemolytic anemia is usually associated with one of two clinical findings: deposition of fibrin within the microvasculature or severe systemic hypertension. Several experimental models have helped to elucidate the mechanisms of red cell fragmentation under these two conditions.

When intravascular coagulation is induced in rabbits by the infusion of endotoxin<sup>74</sup> or thrombin,<sup>76</sup> the onset of red cell fragmentation and hemoglobinemia is closely linked to the development of renal glomerular thrombosis. The hematologic changes can be prevented by pretreatment with heparin. These findings strongly suggest a link between the deposition of platelet and fibrin thrombi and red cell fragmentation. The rate of fibrin deposition appears to be an important variable: rabbits injected with Arvin, a powerful coagulant derived from Malayan pit viper,<sup>129</sup> only develop intravascular hemolysis when rapid defibrination is produced by large amounts of Arvin. Presumably, smaller fibrin deposits are lysed as soon as they are formed; even with large amounts of Arvin, fibrinolysis does not lag far behind and hemolysis stops quickly, usually within minutes after injection of Arvin. If, on the other hand, fibrinolysis is prevented by the prior administration of  $\epsilon$ -aminocaproic acid (see Chapter 38), con-



tinued hemolysis and marked red cell fragmentation persist for many hours.

Histologic studies associate hemolysis with a loose fibrin network to which red cells adhere.<sup>129</sup> Other *in vitro* experiments have expanded these observations: when red cells are forced through a loose fibrin clot within a slide chamber, they attach to very fine fibrin threads and fold themselves around these razor-sharp strands<sup>78</sup> (Fig. 28-2). As other cells flow past their attached fellows they either cause their release or their fragmentation, often with resealing of the membrane. This resealing results in the formation of the oddly shaped fragments described previously. Similar results are obtained when nylon or glass fibers are used in artificial circuits.<sup>78</sup> This suggests that red cell fragmentation depends on the arrest of individual cells by any obstruction of small dimensions in relation to its own size, with subsequent injury of the arrested cell by the rapidly flowing blood stream. Strands or spikes less than one micron in diameter are particularly prone to produce red cell fragmentation.<sup>78</sup>

Other experimental models have dealt with the role of hypertension in producing red cell

fragmentation. When rats are given desoxycorticosterone and a high salt intake they develop malignant hypertension with widespread degenerative vascular lesions, and, coincidentally, a hemolytic anemia characterized by red cell fragmentation.<sup>141</sup> Closely analogous to the human counterpart of this syndrome, the most anemic animals are those with the most severe vascular lesions. While some fibrin and platelet deposits are seen within the lumen of some vessels, they do not play a predominant role in red cell fragmentation. The striking finding on electron microscopy of arterioles is the close juxtaposition of erythrocytes to endothelial cells, the apparent molding of projections of endothelial cells around the erythrocytes, and the partial penetration of the endothelium by erythrocytes. Red cell fragmentation is thought to be the result of shearing stress from the force of the arterial blood as it moves past red cells that are attached to endothelial projections or have partially penetrated the endothelium. Parallel observations were made in rabbits receiving catecholamine infusions<sup>141</sup>; red cells were shown to be squeezed through the endothelium, and it was

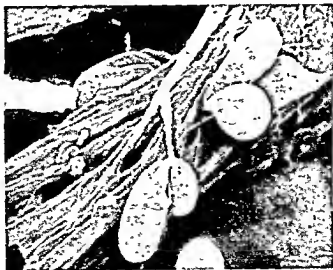


Fig 28-2. Scanning electron micrograph of red cells "clothes lined" over fine fibrin strands, *in vitro* model. Other cells, moving past these trapped erythrocytes may cause their fragmentation. Thicker fibrin strands in background do not cause this injury (From Bull and Kuhn,<sup>77</sup> courtesy of the authors and Henry M. Stratton, Inc.)

suggested that this may be the principal mechanism of red cell fragmentation in this experimental model.

Thus damage to red cells and hemolysis may result either from the intravascular deposition of fibrin or from primary vascular damage produced by severe hypertension or vasoconstriction. In human disease, microangiopathic hemolytic anemia has been found to complicate vascular disease produced by either mechanism. The relative importance of these two, or of other as yet unidentified mechanisms, clearly has a bearing on the nature and effectiveness of therapy, as well as on the ultimate prognosis.<sup>73</sup>

## The Hemolytic Uremic Syndrome

The term "hemolytic uremic syndrome" was first coined by Gasser and his coworkers, in 1955, to describe the fatal association of an acute intravascular hemolytic anemia and renal failure in infants and young children.<sup>91</sup> These authors were also the first to recognize the presence of fragmented erythrocytes ("zerfallene Formen") in at least one of the patients studied. Several hundred patients, some in large series,<sup>93,94,104,111</sup> have been reported since then and the clinical features of the disease are now well defined. Nevertheless, there still is some uncertainty about the cause and pathogenesis of this syndrome and, consequently, the optimal approach to therapy.

### Clinical Manifestations

The hemolytic uremic syndrome characteristically appears suddenly in otherwise healthy infants and children. The highest incidence is in the first year of life, with a peak onset at the age of seven months.<sup>72</sup> After the first year of life, there is a marked decline in the number affected, but sporadic cases have been reported among late teenagers and young adults. The most common antecedent illness is gastroenteritis, but in some patients the onset of anemia and

renal failure is preceded by a brief febrile illness, usually of undetermined origin. Sometimes bacterial,<sup>87,132,133</sup> rickettsial,<sup>116</sup> or viral<sup>193,96,123,127</sup> pathogens have been identified. The occurrence of outbreaks of the disease suggests that particular viruses may sometimes be responsible.<sup>68,93,115,130</sup> In some subjects the hemolytic uremic syndrome has followed immunization against diphtheria, pertussis and tetanus,<sup>85,89,122</sup> polio,<sup>120</sup> measles,<sup>109</sup> or smallpox.<sup>117</sup>

The onset of hemolysis and renal failure may be dramatic with sudden pallor, abdominal pain, vomiting, and the appearance of dark-red or nearly black urine. These manifestations quickly give way to oliguria and, in many patients, total anuria. On *physical examination* the infant usually is pale, slightly jaundiced, and may have purpuric lesions, ecchymoses, and evidence of bleeding from mucous membranes, especially if aspirin has been given during the prodromal illness. Moderate hepatomegaly is common, but splenomegaly is not; the kidneys may be palpable and tender. Various neurologic manifestations may be noted, including drowsiness, convulsions, and transient pareses, but these are not as frequent as in patients with thrombotic thrombocytopenic purpura. An elevated blood pressure is present in about half the patients and helps to differentiate this syndrome from other causes of acute renal failure associated with diarrhea, such as dehydration and renal vein thrombosis.

### Laboratory Findings

The *anemia* often is severe and may be accompanied by moderate polymorphonuclear *leukocytosis*. *Thrombocytopenia* may be marked, but is not invariably present. The lowest platelet counts usually are found in the most anemic patients. The blood smear is distinctive; many fragmented erythrocytes, burr cells, and some microspherocytes contrast with a moderate number of large polychromatophilic cells representing reticulocytes. No intracorpuseular defects are evident and the extraerythrocytic nature of the he-

molytic process may be demonstrated by the markedly shortened survival of erythrocytes from normal donors. The reactions to both the direct and indirect antiglobulin tests are negative, but a few exceptions have been noted.<sup>81,99</sup>

The plasma contains free hemoglobin that is frequently detectable by naked-eye examination. Hemoglobinemia may be marked and correlates with the degree of anemia and the severity of the morphologic changes. Haptoglobins are low or absent and methemalbumin may be detectable spectroscopically. The bilirubin levels usually are only mildly elevated and rarely exceed 2 to 3 mg/dl. The blood urea nitrogen and serum creatinine levels may be very high.

The urine usually contains hemoglobin and hemosiderin in addition to large quantities of other proteins, especially albumin. Microscopically, red cells, white cells, and casts are seen.

In a few patients, prolonged prothrombin times and partial thromboplastin times, low levels of labile clotting factors, and elevated levels of fibrin breakdown products have been found,<sup>84,107,118,123,133,144</sup> but usually the levels of factors V, VIII, and fibrinogen are normal or increased.<sup>66,93,102,110,139</sup> Some workers have demonstrated an increased <sup>131</sup>I-fibrinogen turnover rate, but this has been denied by others.<sup>116a</sup> There is no demonstrable increase in the deposition of <sup>131</sup>I-fibrinogen in the kidneys.<sup>116a</sup>

### Pathologic Changes

The main lesions are found in the kidney,<sup>143</sup> and specifically in the renal glomeruli, some or all of which may be affected. On gross examination the kidneys are swollen and pale, with many "flea-bite" hemorrhages on the surface. Light microscopy confirms the focal nature of the lesions, both between and within individual glomeruli. In the most severely affected glomeruli, these lesions consist of congestion and infarction with hyaline thrombosis of the capillaries. Less affected glomeruli show thickening of capillary walls by eosinophilic, faintly PAS-positive hyaline

material that is deposited between the endothelial cells and the basement membrane, and hypertrophy and proliferation of mesangial endothelial cells. On electron microscopy,<sup>81,143</sup> granular or fibrillar electron-dense material is seen both within the endothelial cells and in the space between the endothelial cells and the basement membrane. Large numbers of platelets also have been identified within the glomerular capillaries.<sup>81</sup> There is evidence to suggest that the material deposited in the glomerular capillary wall consists, at least partially, of fibrin or fibrin derivatives,<sup>81,82,88</sup> as does the hyaline material found on electron microscopy within the endothelial cells.

The afferent glomerular arterioles, and sometimes the interlobular arteries, show fibrinoid necrosis of their walls. Foci of renal cortical necrosis are frequent in severely affected patients.

### Etiology and Pathogenesis

It has been suggested that overt or occult intravascular coagulation may be a central factor in the development of the microangiopathy, and that focal intravascular coagulation may be the cause of the hemolysis and uremia.<sup>72,107,140</sup> Furthermore, it is held that intravascular coagulation may be accelerated by the release of thromboplastic materials from intravascularly lysed erythrocytes.<sup>71</sup> In support of this view, the beneficial effects of early treatment with heparin<sup>68,72,84,95,103,107,118,119</sup> and/or streptokinase<sup>119</sup> have been cited. The hemolysis is attributed to mechanical damage to red cells as they pass over fibrin deposits or projections of abnormal endothelial cells, as discussed earlier in this chapter (page 938). The thrombocytopenia is attributed to the aggregation of platelets in glomerular lesions and to their consumption during the process of low-grade intravascular coagulation observed in some patients.<sup>67</sup> Supporting this view is the observation that, following anticoagulation with heparin, the platelet count usually rises.

The hypertrophy and hyperplasia of phagocytic glomerular epithelial cells appear to be

due to the deposition of fibrin. This is suggested by the presence of hyaline material within endothelial cells and by animal experiments in which intravascular coagulation within the capillaries was triggered by immunologic means.<sup>140</sup> Endothelial proliferation may be so severe as to contribute significantly to the poor glomerular circulation. It is preventable by anticoagulation, which removes the primary cause of the cellular proliferation.<sup>140</sup>

The factors precipitating the development of vascular lesions in children with hemolytic uremic syndrome remain obscure. The association of the syndrome with viral and bacterial infections, and immunizations, as well as the frequent occurrence of this disorder in patients with immune deficiency syndromes,<sup>85 89 93,105</sup> suggests that immunologic reactions, perhaps of a very specific type, may play an etiologic role. Since the pattern of specific immune responses appears to be genetically determined,<sup>112</sup> the occurrence of the hemolytic syndrome in siblings and close relatives,<sup>65,80 93 110</sup> often at intervals of some months or years, is of special interest. It is possible that this pattern reflects an inherited peculiarity, though not necessarily an abnormality, of the immune response.

Soluble immune complexes have been shown to initiate intravascular coagulation in vitro<sup>128</sup> and to produce renal glomerular thrombi in vivo.<sup>104</sup> In experimental models the development of glomerulonephritis can be shown to be associated with "incomplete" immune responses.<sup>92,124</sup> This observation has been proposed as an explanation for the low age incidence of the hemolytic uremic syndrome,<sup>72</sup> as well as the frequent occurrence of the condition in patients with various immune-deficiency syndromes.

## Therapy

At least some of the recent decrease in the mortality of the hemolytic uremic syndrome reflects better general care of patients in renal failure and is not attributable to any specific form of therapy.<sup>110</sup> In isolated cases, for in-

stance, peritoneal dialysis has allowed patients to recover spontaneously after two or more weeks of anuria.<sup>133,136</sup> Similarly, the astute management of fluids and electrolytes, blood transfusions, and the control of hypertension undoubtedly contribute to the survival of patients who are capable of recovering spontaneously if they are given sufficient time.

Many patients have been treated with *heparin*.<sup>68,72,84,93,100,103,107,110,118,119,133,141</sup> No well-controlled trials are available, but the effect of this therapy has often been dramatic, with prompt fall in plasma hemoglobin concentrations, increased platelet count, and improvement in renal function. Failures of heparin therapy have been reported.<sup>93,101a,116a,142</sup> In some patients the dose of heparin may have been inadequate since it has been shown that the amount of heparin needed to bring about the desired effect is larger in patients with microangiopathic hemolytic anemia than in those with other conditions.<sup>72</sup> We have found that minimum doses of 25 U/kg/hr have to be administered and frequently doses of 35 U/kg/hr are needed in order to produce significant prolongation of the clotting time, or to achieve a satisfactory plasma level as measured by protamine neutralization. If the clotting time is prolonged prior to therapy because of ongoing intravascular coagulation or for other reasons, it cannot be used as a guide to the adequacy of therapy. In some centers, heparin is never used.<sup>137</sup>

Theoretically, fibrinolytic agents should give good results, and favorable responses to streptokinase, usually in conjunction with heparin, have been reported.<sup>69,119</sup> Complications, especially bleeding, are common with these agents (Chapter 38) and controlled trials are not available.

*Adrenocorticoids* have been given to many patients, occasionally with apparent benefit,<sup>123</sup> but most authors are not convinced of the value of this form of therapy.<sup>68 93,100,101 110,126</sup> Indeed, it has been suggested that steroids may be harmful in patients with intravascular coagulation disorders.<sup>70 110</sup>

## Prognosis

In one study the overall mortality of 250 infants and children with the hemolytic uremic syndrome, who were cared for between 1957 and 1967, dropped from 23% among the first 150 patients to 5% in the last hundred.<sup>93</sup> This probably reflected recognition of milder forms of the disease as well as improved care. Unfavorable prognostic factors include (1) the severity of the acute phase of the disease,<sup>93</sup> (2) the duration of anuria,<sup>93</sup> and (3) the extent of glomerular damage<sup>72</sup>; 80 to 100% of glomeruli are affected in patients who die, whereas 50% or fewer glomeruli have been involved when renal biopsy specimens were obtained from children who recovered.

## Thrombotic Thrombocytopenic Purpura (TTP) (Moschkovitz Syndrome<sup>174</sup>)

Thrombotic thrombocytopenic purpura is a serious but uncommon disorder of young adults. It is characterized by hemolytic anemia, thrombocytopenia, neurologic signs, renal disease, and fever. The clinical manifestations are caused by widespread disease of small blood vessels and appear to merge pathologically and clinically with those of the hemolytic uremic syndrome of infancy and childhood. There are, however, important *clinical differences*: TTP is most frequently seen in young adults, it has a tendency to involve more organ systems, and has a much higher mortality rate than the hemolytic uremic syndrome. Several hundred cases of the disease have been reported and the pertinent clinical data have been summarized in a number of reviews.<sup>152,159-185,188</sup>

## Clinical Manifestations

Although TTP may occur in the very young<sup>171</sup> and the very old, most patients are between 10 and 40 years of age, with a peak incidence in those in the third decade of life. Females are more frequently affected than males (3:2). The chief clinical manifestations

in a large series of subjects are summarized in Table 28-2. It is obvious that virtually all patients have fever, hemorrhagic manifestations, anemia, neurologic signs, and renal disease.

The cause of the fever is not known but it does not appear to be due to superimposed infections. *Hemorrhagic manifestations* usually take the form of petechiae and ecchymoses of the skin, but retinal hemorrhages, epistaxis, bleeding from the gastrointestinal tract, and gross hematuria also are frequently present. Virtually all patients (96%)<sup>152</sup> have *thrombocytopenia*. Anemia is a constant feature and many patients are clinically jaundiced.

*Neurologic manifestations* constitute the initial symptom in about 50% of these patients. The most common presenting signs are mental changes such as confusion, delirium, and altered states of consciousness, or hemiparesis.<sup>185</sup> Other findings include aphasia, seizures, hemisensory changes, ataxia, and field defects. While it is generally stated that the neurologic abnormalities are typically remittent and subject to sudden change,<sup>152</sup> it was found that fewer than half of all patients showed significant improvement from their initial neurologic defects.<sup>185</sup> The frequency of the neurologic manifestations can be attributed to the common and

Table 28-2. Clinical Manifestations of Thrombotic Thrombocytopenic Purpura (TTP)

	Cases	Percent
Fever	237/243	98
Pallor or anemia	246/256	96
Hemorrhagic manifestations	241/251	96
Neurologic manifestations	250/271	92
Renal manifestations	191/217	88
Jaundice	113/271	42
Fatigue malaise	92/271	34
Nausea and vomiting	69/271	25
Abdominal pain	36/271	13
Chest pain	21/271	8
Arthralgia or myalgia	18/271	7

From Amorosi and Ultman<sup>152</sup> courtesy of the authors and Williams & Wilkins

striking involvement of the small vessels of the cortical gray matter and the brain stem.

*Renal disease* is present in most patients and is due to hyaline occlusion of capillaries and arterioles,<sup>88</sup> and proliferative changes within the glomeruli.

Other clinical manifestations include abdominal pain due to pancreatitis<sup>174a</sup> or occlusion of visceral vessels, hepatomegaly (25%), and splenomegaly (20%).<sup>152</sup> Occasionally, Raynaud's phenomena are found.

### Laboratory Findings

The hemoglobin is below 10.5 g/dl in virtually all subjects, and less than 5.5 g/dl in about a third. There is an appropriate reticulocytosis in most of the patients, and normoblasts are frequently found in the blood. The most characteristic morphologic abnormalities are the bizarre, distorted, and fragmented red cells (schistocytes, Fig. 28-1). The reaction to Coombs' test is negative.

Occasional prolongation of prothrombin time and decreased fibrinogen levels have been noted and increased titers of fibrin breakdown products have been reported,<sup>162, 165, 168, 172</sup> but in most patients there is no evidence of disseminated intravascular coagulation.<sup>152, 165, 168</sup> *Thrombocytopenia* is an almost constant finding; platelet counts of 10 to 120  $\times 10^9/l$  were reported in 216 of 224 patients collected in one study.<sup>132</sup> The reaction to the tourniquet test usually is positive, the bleeding time is prolonged, and clot retraction is poor. Increased utilization, rather than decreased production, is the probable cause of the thrombocytopenia.

*Leukocytosis* is present in about one half of all these patients, and some have white blood cell counts in excess of 20.0  $\times 10^9/l$ .<sup>152</sup> In addition, a considerable "left shift" may be seen in the blood, with the appearance of immature granulocyte precursors.

The bone marrow shows erythroid hyperplasia and a normal or increased number of young megakaryocytes with smooth borders.

Elevated unconjugated bilirubin levels are found in most patients and may be accom-

panied by elevations in plasma hemoglobin and decreased levels of haptoglobin, suggesting intravascular destruction of red cells as the most likely cause of the hemolysis. Cross-transfusion studies confirm the extracorporeal nature of the defect leading to hemolysis.<sup>183</sup>

Electroencephalographic and electrocardiographic abnormalities have been reported.<sup>152</sup>

### Pathologic Changes

At postmortem examination, characteristic capillary arteriolar occlusions may be seen in the heart, brain, kidneys, pancreas, and adrenal glands, and, to a lesser extent, in virtually all other organs of the body.<sup>152, 168, 170</sup> The nature of the occluding material has not been completely defined, but fibrin components<sup>82, 89</sup> have been identified by immunofluorescent techniques. Other prominent changes include subintimal deposition of similar material,<sup>88, 163</sup> as well as proliferation of endothelial cells.<sup>163</sup> Some authors regard the specific location of vascular lesions at arteriocapillary junctions, with aneurysmal dilatation of weakened vessel walls, as being characteristic of thrombotic thrombocytopenic purpura,<sup>168, 175</sup> but others have found similar lesions in patients with disseminated intravascular coagulation (DIC).<sup>190</sup>

### Etiology and Pathogenesis

Although multiple small thrombi are responsible for the clinical manifestations of TTP, the reason for their development is totally obscure. There is little laboratory evidence to support the contention that the thrombi are manifestations of a DIC syndrome. Some investigators have demonstrated disseminated intravascular platelet aggregation, rather than thrombi, and claim that this may be the cause of vascular obstruction.<sup>171b</sup> Focal damage of vessel walls may be the primary event and intravascular thrombi and arteriolar aneurysms may be secondary phenomena.<sup>168</sup> The cause of the vessel wall damage is equally obscure. Associated drug allergies,<sup>152</sup> rickettsial

infections,<sup>116</sup> vaccinations,<sup>156a</sup> and autoimmune disorders such as lupus,<sup>154,167,169,184</sup> Sjogren's syndrome,<sup>187</sup> polyarteritis,<sup>75,189</sup> and other forms<sup>172</sup> have been suggested as the underlying causes of the vascular pathologic state, but these associations are rare. Positive reactions to serologic tests for syphilis were obtained in nine of 73 patients; in five of these the reactions may have been biologically false positive ones.<sup>152</sup> The simultaneous occurrence of fatal TTP in a married couple<sup>191</sup> and in two sisters who shared the same house<sup>177</sup> strongly suggests an environmental influence.

### Therapy

Whatever their pathogenesis, multiple small thrombi are responsible for the clinical manifestations of TTP in most cases and rational therapy requires an attempt to block their formation. *Heparin* has been used for this purpose, but it has yielded conflicting results. Both success<sup>150,158,179,180</sup> and failure<sup>149,157,158,165</sup> have been reported. Patients who appear to have benefited have usually received other medications concurrently. No controlled trials are available and it is therefore impossible to judge the true efficacy of heparin.

*Dextran* coats vascular surfaces<sup>156</sup> and platelets<sup>181</sup> and interferes with platelet aggregation, as judged by prolongation of bleeding time.<sup>166</sup> The use of dextran has been reported to have been attended with *apparent* success in the therapy of purpura fulminans<sup>176</sup> and TTP,<sup>168</sup> but these reports need confirmation. Low molecular weight dextran,<sup>158</sup> in contrast to clinical dextran,<sup>168</sup> was of no benefit to one patient with TTP.

If platelet aggregation is the cause of the vascular occlusions,<sup>174b</sup> therapy with drugs which inhibit platelet aggregation, such as aspirin and dipyridamole (Chapter 9), may be of benefit. Success has been reported in a few patients.<sup>151</sup>

Large doses of *adrenocortical steroids* have been used in the therapy of TTP, but did not, by themselves, appear to alter the course of the disease. A review of 46 patients so treated

revealed that only nine of these were still alive at the time of the review.<sup>152</sup>

It has been suggested that *splenectomy*, especially in combination with steroid therapy, may give much better results than either treatment alone; thus, in one series, five of seven moribund patients responded dramatically to this form of therapy,<sup>153</sup> although two subsequently died in a relapse. Others have also reported good success with splenectomy and steroids.<sup>152,164,182,183</sup>

### Prognosis

TTP is a most serious disease and usually runs a rapidly progressive and fatal course. Most patients (80%) are dead within three months, and fewer than 10% survive for more than a year.<sup>152</sup> In actual fact the number of patients who survive an attack of TTP may be much lower, since many of the long-term survivors have been shown *not* to have classical TTP. Furthermore, it is likely that more successes than failures are reported in the medical literature.

## Disseminated Intravascular Coagulation (DIC)

Microangiopathic hemolytic anemia also is associated with a variety of disorders characterized by disseminated intravascular coagulation (Chapter 38), including that associated with sepsis,<sup>238,239</sup> purpura fulminans,<sup>218</sup> heart stroke,<sup>245</sup> and abruptio placentae.<sup>67,201</sup> In all of these clinical conditions, red cell fragmentation appears to be due to the deposition of fibrin within the microvasculature. Fortunately, hemolysis often is not severe and may not contribute significantly to the morbidity of the disease. As the underlying disease comes under control with appropriate therapy, the fragmentation of red cells also ceases.

## Microangiopathy Associated with Immune Mechanisms

Microangiopathic hemolytic anemia may also be a feature of diseases in which the

microvasculature is damaged by immune mechanisms. This includes lupus erythematosus,<sup>75</sup> acute glomerulonephritis,<sup>75,99</sup> and the homograft reaction,<sup>213</sup> all of which are characterized by the presence of antigen-antibody complement complexes within selected areas of the microvasculature.<sup>223,229</sup> Red cell fragmentation also has been observed in association with polyarteritis nodosa,<sup>75,139</sup> scleroderma,<sup>210</sup> and Wegener's granulomatosis.<sup>207</sup> While the immunologic nature of the latter diseases is as yet poorly defined<sup>211</sup> this is, nevertheless, likely. Fibrin deposits have been demonstrated in the vascular lesions of patients with these diseases.<sup>216,234</sup>

Antigen-antibody-complement complexes readily trigger the coagulation cascade,<sup>140</sup> probably through an effect on platelets.<sup>193</sup> As a result, fibrin is deposited and this stimulates the proliferation of endothelial cells.<sup>140</sup> It is likely that both the fibrin deposition and the endothelial changes are responsible for the red cell fragmentation (see page 938).

Microangiopathic hemolytic anemia has also been reported in association with *systemic amyloidosis* (Chapter 53).<sup>221</sup>

## Giant Hemangiomas and Hemangioendotheliomas

Microangiopathic hemolytic anemia has been described in patients with giant hemangiomas<sup>220,237</sup> and in those with hemangioendotheliomas of the liver.<sup>195</sup> There is good evidence that local coagulation in the abnormal blood vessels plays a role in the red cell fragmentation.<sup>220</sup> Irradiation of the hemangioma has been found to abolish red cell fragmentation and the associated thrombocytopenia.<sup>220</sup>

## Disseminated Carcinoma

Microangiopathic hemolytic anemia has been observed in a large number of patients with disseminated malignant disease involving the stomach,<sup>75,202,214,227,243</sup> colon,<sup>214</sup> pancreas,<sup>214</sup> breast,<sup>202</sup> lung,<sup>75,202</sup> pros-

tate,<sup>75</sup> and other sites.<sup>214</sup> On the basis of coagulation studies,<sup>222,227,243</sup> many of these patients were thought to have intravascular coagulation and some were shown to have markedly increased rates of fibrinogen catabolism.<sup>67</sup> In other patients the red cell fragmentation was attributed to widespread occlusion of the pulmonary vasculature by tumor emboli, but this mechanism, if indeed operative, would not account for the red cell fragmentation found in a majority of these patients. Among 12 patients with microangiopathic hemolytic anemia that occurred in association with metastatic carcinoma originating in the stomach, breast, or lung, tissues were available for study from 11 and all 11 were found to have mucin-secreting adenocarcinomas.<sup>202</sup> It was suggested that mucin was directly responsible for the induction of intravascular coagulation by mechanisms that had been demonstrated *in vitro*. Of these 12 patients, 10 had been thrombocytopenic, all had greatly increased rates of fibrinogen catabolism, and fibrin breakdown products were found in the serum. In addition, hyaline thrombi were demonstrated in various tissues from several of these patients. It was suggested that the microangiopathic hemolytic anemia was secondary to intravascular coagulation and this in turn was brought about by thromboplastic substances released by tumor cells.<sup>202</sup>

## Eclampsia and Preeclampsia

Acute intravascular hemolysis has long been known to complicate the clinical course of patients with eclampsia and severe preeclampsia.<sup>236</sup> More recently, red cell fragments have been observed in such patients.<sup>242</sup> Hypertension may itself lead to red cell fragmentation and hemolysis by mechanisms discussed earlier (page 939), but microangiopathic hemolytic anemia has been shown to precede the development of hypertensive retinopathy in some subjects,<sup>200</sup> and therefore factors other than hypertension may be important. The deposition of fibrin in endothelial cells or between the endothelial cells and the basement membrane suggests that intra-



vascular coagulation plays an important role in the renal pathologic state of preeclampsia<sup>217,231,238</sup> and, hence, in the pathogenesis of red cell fragmentation. The frequent occurrence of thrombocytopenia and cryofibrinogenemia in preeclamptic patients has been cited as evidence for a low-grade process of intravascular coagulation.<sup>200</sup> Pregnancy also has been observed to render the experimental animal more susceptible to the development of the generalized Schwartzman reaction.<sup>232,233,249</sup> Gamma globulin and complement have not been demonstrated within the fibrin deposits,<sup>231</sup> however, and the reason for the intravascular coagulation in these patients remains obscure.

In one review of 14 patients with preeclampsia and intravascular hemolysis<sup>200</sup> the hemoglobin was found to range between 4.2 and 10.5 g/dl (median 6.5 g/dl) and all patients were thrombocytopenic (7 to  $120 \times 10^9/l$ , median 52). The reticulocyte count was inappropriately low in several patients. Pigmentary evidence of intravascular hemolysis was reported in nine, and fragmented red cells were noted in six. Fibrinogen levels were measured in six patients and were found to be normal in five and nearly normal in another. In nine of the 14 patients the diastolic blood pressures were above 100 mm Hg, but in five significant hemolysis occurred in the absence of elevated pressures (130/80 to 160/90). Furthermore, in some patients with hypertensive retinopathy, hemolysis began while the fundi were still normal. All patients had proteinuria and increased blood urea levels. The maternal mortality rate was 64%, and the perinatal mortality was 44%. Only one patient has been reported in whom the hemolysis and thrombocytopenia decreased when heparin therapy was given.<sup>200</sup>

### Malignant Hypertension

An association between red cell fragmentation and malignant hypertension was first recorded by Dacie in 1954,<sup>73</sup> and has since then been confirmed in many other studies.<sup>75,197,205,226,241</sup> Red cell fragmentation

was found in 16 of 24 patients with malignant hypertension, and 10 of these patients also had platelet counts of  $100 \times 10^9/l$  or less.<sup>226</sup> None of these changes was seen in 63 patients with hypertension in the absence of retinal hemorrhages and exudates.

The pathogenesis of microangiopathic hemolytic anemia in malignant hypertension is poorly understood. Most authors attribute the hemolysis to the presence of fibrinoid necrosis within the arterioles,<sup>73</sup> which in turn appears to depend on the presence of hypertension. It has been suggested that subsequent hemolysis may aggravate the arteriolar lesions by producing additional intravascular coagulation.<sup>199,226</sup> Alternatively, the deposition of fibrin, aided by the clot-promoting properties of lysed red cells, may itself underlie the pathogenesis of the malignant phase of hypertension.<sup>226</sup> However, red cell fragmentation is not a constant feature of malignant hypertension,<sup>226</sup> and the fragmentation of red cells may be brought under control by the simple expedient of lowering the blood pressure appropriately,<sup>197</sup> thereby suggesting that hypertension is the cause, rather than the result, of the red cell fragmentation.

### March Hemoglobinuria

March hemoglobinuria is a hemolytic disorder in which transient hemoglobinemia and hemoglobinuria develop in susceptible individuals after strenuous exercise of a kind that involves forceful contact of the body with a hard surface. While red cell fragmentation is not seen, the condition carries all the hallmarks of acute intravascular hemolysis, which presumably is due to the mechanical disruption of circulating red cells. The condition appears to be relatively rare: reports of fewer than 100 cases have been published since Fleischer<sup>256</sup> described the first case in 1881,<sup>253</sup> but clinically inapparent hemoglobinemia may be much more common.<sup>259</sup>

### Clinical Manifestations

With rare exception,<sup>257,261</sup> march hemoglobinuria is confined to males,<sup>253</sup> most com-

monly in those in their late teens or early twenties. The age and sex distribution may reflect the more frequent participation of young males in severe and prolonged exertion. In most subjects, hemoglobinuria is precipitated by prolonged marches or competitive running, but the syndrome has also been described in conga drum players,<sup>262</sup> following karate exercises,<sup>266</sup> and in an individual suffering from dementia praecox who had cultivated the unusual habit of slapping his forehead violently for periods of 20 to 30 minutes.<sup>255</sup>

Passage of red or dark urine after physical exertion is often the only complaint. Occasionally there may be nausea; vague pains in the abdomen, back, or thighs; or a burning feeling in the soles of the feet. Hemoglobinuria characteristically occurs immediately after exercise and lasts for a few hours only. It is most common at the beginning of an athlete's running career or on resumption of road training.<sup>253</sup> Attacks may recur for several weeks or months, rarely for a few years, but may also remit spontaneously despite continuing exercise.

Clinical findings are usually insignificant. Mild transient jaundice is rarely present, even immediately following attacks of hemoglobinuria. Occasionally the liver or spleen is palpably enlarged. On cystoscopy, red-colored urine is seen to issue from both ureteric orifices.<sup>253</sup>

### Laboratory Findings

Anemia is uncommon, as less than 1% of the patient's circulating red cells are hemolyzed in an average paroxysm.<sup>253,264</sup> The blood smear does not contain fragmented red cells,<sup>253</sup> although polychromatophilia and a mild reticulocytosis may follow repeated hemolytic episodes. Reactions to osmotic and mechanical fragility tests are normal.<sup>259</sup>

The hallmarks of intravascular hemolysis include a raised serum hemoglobin value, lowered serum haptoglobin, and sometimes methemalbuminemia. The bilirubin rarely exceeds 2 mg/dl. Serum lactic dehydrogenase levels may be elevated.<sup>261</sup> The urine contains

hemoglobin, and after recurrences it may contain hemosiderin. Albuminuria and abnormalities of the urine sediment have been noted,<sup>264,264</sup> but permanent renal impairment is rare; acute tubular necrosis has been reported in one patient.<sup>263</sup>

### Differential Diagnosis

March hemoglobinuria must be differentiated from myoglobinuria (Chapter 20) and from other causes of hemoglobinuria such as paroxysmal cold hemoglobinuria (Chapter 27) and paroxysmal nocturnal hemoglobinuria (Chapter 29). In the latter, hemoglobinuria may be aggravated by exercise. The red color of the urine may suggest porphyria (Chapter 32).

### Etiology and Pathogenesis

The cause of the intravascular hemolysis is not entirely clear, but several observations of interest have been made. (1) Hemoglobinuria usually follows exercise on a hard floor or roadway and does not occur when the same individual uses a grass or cinder track or engages in other strenuous exercise such as swimming or cycling.<sup>258</sup> (2) Patients suffering from march hemoglobinuria frequently have a very heavy stride. (3) Hemoglobinuria can be prevented by the use of shoes with thicker and more resilient soles than those usually worn.<sup>251</sup> Thus it was postulated that susceptible individuals destroyed red cells in the soles of their feet while running.<sup>253</sup> (4) Confirmation came from the ingenious experiments of Davidson<sup>252</sup> who inserted polyvinyl tubes containing blood into the running shoes of susceptible individuals and showed that they destroyed their own and control blood at approximately the same rate, and to a much greater degree than control subjects running on the same surface. Whether hemolysis is entirely attributable to the type of exercise and the style of running or whether other factors make the red cells of some people particularly susceptible to hemolysis is not clear. It has been suggested that susceptible individuals may suffer from abnormal

hemolysis with ordinary activity as well,<sup>250,264</sup> but no specific cellular or vascular defects have been identified.

The self-limited nature of the attacks has been attributed to the increased mechanical fragility of old erythrocytes.<sup>265</sup> Following elimination of susceptible cells, the rate and degree of destruction of the remaining younger cells may be too low to cause further hemoglobinuria.<sup>251</sup>

## Therapy

No specific therapy is available. Attacks may be prevented by wearing shoes with more resilient soles and by changing to a less traumatic running style.

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## Paroxysmal Nocturnal Hemoglobinuria (PNH)

Etiology and Pathogenesis  
 Clinical Manifestations  
 Laboratory Findings  
 Differential Diagnosis  
 Treatment  
 Course and Prognosis

**P**AROXYSMAL nocturnal hemoglobinuria, also known as the Marchiafava-Micheli syndrome, is an uncommon disorder of insidious onset and chronic course. It is characterized, in the classic case, by attacks of intravascular hemolysis and hemoglobinuria, which occur chiefly at night. In a majority of patients, however, the classic pattern is not observed; in these, PNH may be manifested by chronic intravascular hemolysis without a distinct nocturnal pattern, or by pancytopenia, iron deficiency, or recurrent thrombotic episodes. Hemolysis in PNH occurs because of an obscure structural defect of PNH red cells which makes them unusually susceptible to the lytic action of complement.

An excellent account of PNH was given by Strubing in 1882<sup>130</sup>; the disease was subsequently described by Marchiafava and Nazari in 1911 and by Micheli in 1931.<sup>122</sup> By 1953 some 162 cases had been collected in the medical literature.<sup>20</sup> Undoubtedly many cases are overlooked, for the atypical forms are relatively common.

### Etiology and Pathogenesis

Cross transfusion studies have established that PNH is due to an intrinsic abnormality of the red cells,<sup>19</sup> since normal cells survive normally in patients with PNH, while PNH erythrocytes have a shortened life span within the patient or in a normal recipient. Not all PNH red cells are equally susceptible to hemolysis, however, and distinct cohorts of relatively long-lived and very short-lived cells can be readily distinguished by red cell survival studies.<sup>99,117,118</sup> The relative proportions of complement-sensitive and therefore short-lived cells and of complement-insensitive cells vary considerably from patient to patient, and there is good correlation between the numbers of such cells and the clinical course of each patient.<sup>58</sup>

The existence of cell populations with varying sensitivity to lysis in acid or by complement-mediated mechanisms can also be demonstrated by *in vitro* tests.<sup>43,58,115,116</sup> In addition to normal cells and markedly sensitive ones, *in vitro* studies have defined a population of cells with intermediate sensitivity.<sup>115</sup> The majority of patients have either markedly complement-sensitive and normal cells, or markedly complement-sensitive cells and cells of intermediate sensitivity; a smaller number of patients have all three types of cells or normal and intermediate cells only (Fig. 29-1).<sup>115,116</sup> For lysis to occur, complement-sensitive cells require only one twenti-



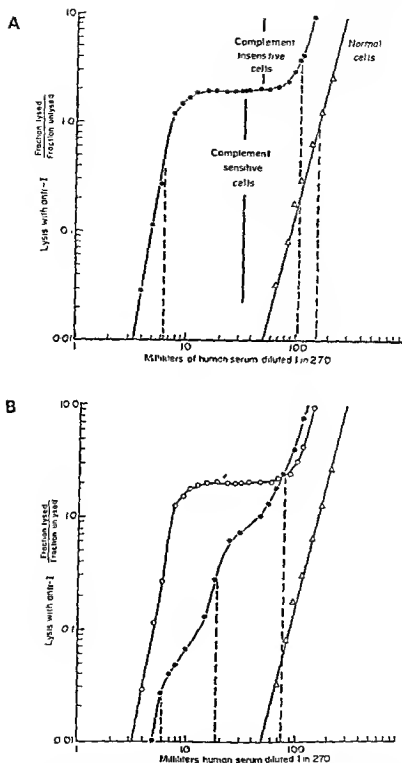


Fig. 28-1. Sensitivity of antibody (anti I) coated red cells to complement mediated lysis, using diluted human serum as a source of complement. The 50% lysis point for each population of cells is indicated (broken lines). A,  $\Delta$ — $\Delta$  Normal donor,  $\bullet$ — $\bullet$  PNH patient with two populations of cells, complement sensitive and insensitive. B,  $\Delta$ — $\Delta$  Normal donor,  $\bullet$ — $\bullet$  PNH patient with three populations of cells, complement sensitive, insensitive, and intermediate.  $\circ$ — $\circ$  PNH patient from A superimposed. (From Rosse,<sup>115a</sup> courtesy of the author and British Journal of Haematology.)

eth the amount of complement needed to disrupt normal cells. Intermediate cells are six to seven times as sensitive as normal cells.<sup>115</sup> The mechanism of erythrocyte lysis by complement is discussed in Chapter 5 (page 203).

Sensitive cells appear to be the product of an abnormal clone of marrow precursor cells<sup>101,115</sup> and do not acquire the defect while circulating.<sup>111</sup> The proportion of abnormal cells is greater in the marrow than in the blood and, among circulating cells, is greatest among young cells, especially reticulocytes.<sup>70,95,99,114,117</sup> If the defect were acquired in the circulation the proportion would be greater among older cells.<sup>115</sup> Erythrokinetic studies have shown that the complement-sensitive population does not gain recruits from the complement-insensitive population.<sup>114</sup> The proportion of sensitive and insensitive cells remains stable for long periods, but population shifts occur during the onset of the disease and in those patients who recover spontaneously.<sup>115</sup>

The increased susceptibility of PNH cells to complement lysis can be demonstrated whether complement activation is induced via the classic complement pathway or via the alternate, properdin-dependent<sup>103</sup> pathway (see Chapter 7, page 333, for a description of both complement pathways). The classic C1-dependent reactions can be induced by antibody,<sup>82,117</sup> whereas lysis in acidified medium (see below) proceeds via the alternate, properdin-dependent pathway.<sup>37,82,115</sup> The alternate pathway also is activated by Naja Naja cobra venom<sup>82</sup> and inulin.<sup>37,82</sup> Lysis induced in media of low ionic strength (see sugar water test and sucrose hemolysis tests, below) probably proceeds via both pathways.<sup>82</sup> The mechanism whereby complement is activated *in vivo* has not been completely delineated but probably involves the alternate pathway predominantly, since neither antibody nor C3 is usually present on circulating cells.<sup>115</sup>

Excessive lysis in acidified human serum is a characteristic feature of PNH cells.<sup>39,41,60</sup> The optimal pH for this reaction is 6.8 to

7.0 with a marked reduction in the degree of hemolysis below or above this range. This contrasts with the much broader range of activity present in antibody-mediated lytic reactions.<sup>60</sup> The optimal temperature for acid hemolysis is between 37° and 40° C; there is a marked reduction in hemolytic activity at higher temperatures due to inactivation of labile hemolytic factors in the serum.<sup>60</sup> Similar to immune hemolytic reactions requiring complement, PNH acid hemolysis is inhibited by small increases in ionic strength and is favored by conditions of low ionic strength. The reaction also requires Mg<sup>++</sup>, but, unlike classic immune hemolytic reactions, is independent of Ca<sup>++</sup>.<sup>87</sup> Co<sup>++</sup> and Mn<sup>++</sup> are the only divalent cations that can substitute for Mg<sup>++</sup><sup>60</sup>; high concentrations of Ca<sup>++</sup> will actually inhibit the reaction.<sup>58,87,138</sup>

The phenomenon of increased susceptibility of PNH cells to hemolysis by *immune mechanisms* has been used for the detection of serum antibodies not demonstrable by other means.<sup>21</sup> This increased sensitivity to antibody-mediated lysis appears to be due to an increased sensitivity to complement rather than an increased reactivity with antibody; since agglutinin titers are the same for PNH and normal cells,<sup>79</sup> approximately the same amount of antibody is bound by both types of cells<sup>60</sup> and the same amount of complement is fixed by normal and PNH cells equally sensitized by antibody.<sup>118</sup>

It has been postulated that the increased sensitivity of PNH cells to complement is related to a *structural defect of the cell membrane*, but the true nature of the defect remains elusive. Certain changes have been revealed by *electron microscopy*<sup>21,80,134</sup> and include a patchy, pitted red cell surface and many cleft-like faults (Fig. 29-2), but it has not been established that these changes reflect the primary corpuscular defect.

The most consistently demonstrated *biochemical abnormality* of PNH erythrocytes is a decrease in membrane acetylcholinesterase (AChE) activity.<sup>27,49,72,93</sup> The deficiency is a regular finding in severe and moderately severe cases<sup>95</sup> and is most pronounced in cells

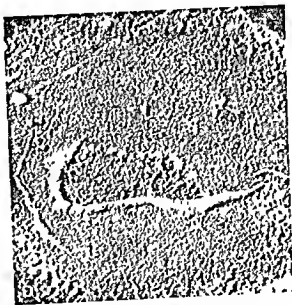
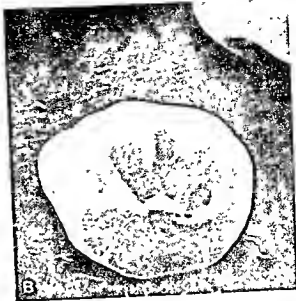
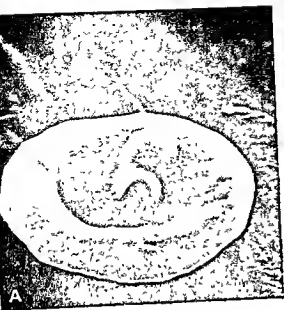


Fig 29-2 A and B Scanning electron micrographs (X11 000) of red cells from patients with PNH. Note protruberances, craters, and pits at site of cell concavity. C and D, Transmission electron micrographs (X10,000) of (C) normal red cell membrane and of (D) PNH like cell (AET treated cell) showing loss of membrane structure. Compare with Figure 3-11 page 94 (From Lewis et al,<sup>41</sup> courtesy of the authors and the Journal of Clinical Pathology)

that are most susceptible to hemolysis *in vivo*.<sup>72</sup> Normally, AChE activity is highest in young erythrocytes and decreases progressively with age, but young PNH cells appear to be as deficient in this enzyme as older cells. Nevertheless, it is unlikely that low AChE levels are directly responsible for the lysis of PNH cells, including reticulocytes,

since inhibition of enzyme activity in normal cells *in vitro* does not lead to increased acid hemolysis,<sup>72</sup> and *in vivo* inhibition of AChE does not influence the red cell survival time.<sup>96</sup> In addition, the enzyme appears to be absent from the red cells of several animal species and this has no apparent ill effect.<sup>47</sup> Since reduced AChE activity accompanies other

insults to red cells such as treatment with proteolytic enzymes,<sup>105</sup> anti-red cell sera,<sup>12</sup> hyperbaric oxygen,<sup>89</sup> or reduced glutathione,<sup>4</sup> it seems likely that reduced AChE activity is a secondary rather than a primary abnormality.

The lipids of the red cell have also received wide attention, but there is no agreement as to whether an abnormality actually exists. Reported changes include a decrease of phosphatidyl choline and an increase in phosphatidyl serine<sup>16</sup>; a decrease in both arachidonic and linoleic acid content<sup>83</sup>; an increase in arachidonic and pentanoic acid and a decrease in the oleic acid content.<sup>87,98</sup> Still other studies report no quantitative differences in phospholipid and fatty acid composition.<sup>76,107</sup> Dietary intake is known to bring about changes in red cell lipids (Chapter 3, page 98) and it is not clear whether adequate dietary controls were adhered to during the reported studies.<sup>76</sup> Nevertheless, there is no doubt that PNH red cells exposed to  $H_2O_2$  form more lipid peroxides and are more readily lysed by  $H_2O_2$  than are normal cells,<sup>91</sup> even though cellular glutathione peroxidase and catalase activities are normal<sup>90</sup> and there is no apparent tocopherol deficiency in PNH. In addition, lipids extracted from PNH erythrocytes are more than normally susceptible to peroxidation by ultraviolet light.<sup>91,94</sup> It has been suggested that PNH red cells may suffer from some basic abnormality of lipid structure which renders them susceptible to excessive peroxidation and membrane damage.<sup>91</sup> Others consider the lipid changes to be secondary to a more fundamental defect.<sup>92</sup>

On the basis of studies in which a PNH-like defect was induced by incubating erythrocytes in alkaline solutions of reduced glutathione (GSH),<sup>4,124,128</sup> some workers have postulated that the primary biochemical lesion of PNH cells may consist of altered SS-SH configurations close to the surface of the cell.<sup>92</sup> To such changes has been attributed the unusual behavior of PNH cells with respect to complement, as well as the lipid abnormalities.<sup>92</sup>

There appears to be no clinically significant abnormality of glycolysis.<sup>7</sup> In addition,

cellular levels of reduced glutathione fall within the normal range and remain stable under stress.<sup>7,90,125</sup> A decreased rate of  $^{32}P$  uptake and release has been reported in the face of a more rapid breakdown of ATP,<sup>157</sup> but these changes are poorly understood and probably do not contribute significantly to the pathophysiology of the PNH erythrocyte.

PNH granulocytes and platelets appear to share the membrane defect of PNH red cells since they also are much more sensitive to lysis by complement or antibodies than are normal cells.<sup>2,35</sup> This observation has led to the suggestion that PNH is the result of a somatic mutation in the primitive stem cell<sup>2</sup> (Chapter 2). The frequent association with aplastic anemia (page 959) suggests that the types of stem cell injury that result in aplasia may, under some circumstances, induce the somatic mutation leading to PNH.

### Clinical Manifestations

Usually PNH begins insidiously. The course tends to be prolonged and constant in any given individual. The diagnosis is made most frequently in the third and fourth decades of life, but occasionally PNH is encountered in childhood<sup>21,25,108,113</sup> or in old age.<sup>21,25</sup> Both males and females are affected. There is no familial tendency and the disease has been described in many racial groups.<sup>25</sup> The illness may range in severity from a mild, clinically benign defect to a chronically debilitating and lethal process.

Most commonly, patients initially complain of weakness, yellowish discoloration of the skin, and other symptoms of chronic hemolysis without obvious hemoglobinuria (Table 29-1). Because PNH is frequently not considered in such patients, the proper diagnosis is often delayed; in one study the average time interval between the onset of symptoms and the correct diagnosis was two and one-half to three years.<sup>21</sup>

The classic presentation of hemoglobinuria or discolored urine is present initially in only a quarter of all patients, and many of these do not suffer from nocturnal exacerbations. Nocturnal hemoglobinuria, when present,

Table 29-1. Presenting Features in 80 Patients with PNH\*

Signs and Symptoms	Number of Patients
Symptoms of anemia	28
Hemoglobinuria	21
Hemorrhagic signs or symptoms	14
Aplastic anemia	10
Gastrointestinal symptoms	8
Hemolytic anemia with jaundice	7
Iron-deficiency anemia	5
Thrombosis or embolism	5
Infections	4
Neurologic signs or symptoms	3

\*From Dacie and Lewis<sup>21</sup> courtesy of the authors and *Series Haematologica*

occurs as the result of an increase in hemolysis during sleep. It is not due to night time per se, since the rhythm can be reversed if the patient stays awake at night and sleeps by day.<sup>22,40</sup> In patients suffering from nocturnal hemoglobinuria, the urine is usually darkly discolored in the morning and clears during the day. However, when hemolysis is intense, hemoglobinuria may persist throughout the day.

The cause of the *nocturnal exacerbation* is poorly understood. Retention of CO<sub>2</sub> with a slight fall in plasma pH has been a popular explanation,<sup>21,39</sup> but avoidance of CO<sub>2</sub> retention by the use of a Drinker respirator failed to prevent the nocturnal sleep rhythm of hemoglobinuria.<sup>14</sup> However, the exacerbation of hemolysis that has been noted to follow strenuous exercise<sup>6,130</sup> is associated with a small reduction in the pH of the blood<sup>6</sup> and it is likely that acidity is even more pronounced in organs with slow blood flow or stasis, thereby providing a suitable environment for increased destruction of PNH erythrocytes.<sup>47</sup> It has also been postulated that nocturnal hemolysis is related to the circadian rhythm in cortisol excretion<sup>43</sup> but this has been questioned by others.<sup>25</sup> Prednisone (30 to 40 mg) does not suppress sleep-related hemolysis in all patients.<sup>43</sup>

In addition to the sleep-related rhythmicity

in hemolysis and hemoglobinuria, most patients suffer from irregularly recurring *exacerbations in hemolysis*, the cause of which is usually unclear. Sometimes exacerbations are precipitated by infections, even minor ones<sup>40,85</sup>; menstruation<sup>21</sup>; transfusions<sup>18,21,40,42</sup>; operations<sup>21</sup>; the taking of iron salts<sup>21,119</sup>; or vaccinations.<sup>122</sup> The attacks of hemoglobinuria are unrelated to cold exposure.

Mild hemolytic episodes often pass without significant symptoms, but more severe attacks may be characterized by substernal, lumbar, or abdominal pain together with drowsiness, general malaise, fever, and headaches. The abdominal pain may be colicky and may last for one or two days. The abdomen may be tender to examination, especially in the left upper quadrant, with guarding and increased symptoms on rebound. The back pain resembles that noted in patients with other types of intravascular hemolysis (Chapters 13 and 27). It is maximal in the lumbar region. Headaches may be severe and sometimes last for several days.

The most serious *complications* include marrow aplasia, thromboses, and infections. The association between *aplastic anemia* and PNH<sup>24,78</sup> has been documented in at least three distinct circumstances: in familial aplastic anemia (Fanconi's),<sup>23</sup> in aplastic anemia likely to have been of drug origin,<sup>24,86,110,121,123,133</sup> and in cases of unknown etiology.<sup>78,132</sup> Indeed, many patients have been followed for prolonged periods with a diagnosis of aplastic anemia and, since they have had few of the obvious changes usually associated with hemolytic disease, the diagnosis of PNH has not been made until the appropriate tests on the patient's red cells (see below) were carried out. In one series of 80 patients, aplastic anemia was the first diagnosis in 23 cases.<sup>25</sup> Less commonly the diagnosis of hemolysis is made first and pancytopenia develops subsequently.<sup>25</sup>

PNH is associated with a striking *predisposition to intravascular thromboses*<sup>106</sup> especially within the venous circulation. These account for about 50% of all deaths in patients with PNH. Fatal thromboses usually

involve the portal system,<sup>14,106</sup> or the brain,<sup>67</sup> but thromboses are also common in the extremities or elsewhere.<sup>106,122,136</sup>

*Abdominal pain* (see above) should always be considered secondary to intra-abdominal thrombosis until proven otherwise. Thrombosis may occur within the portal or mesenteric veins, but progressive, diffuse *hepatic venous thrombosis* (HVT) appears to be particularly common and usually runs a rapidly fatal course,<sup>106</sup> most patients dying within a few months of the onset of this complication. The clinical manifestations include, in addition to abdominal pain, nausea, a sudden increase in liver size (though HVT may occur in the absence of hepatomegaly), and, late in the disease, ascites, often of sudden onset.<sup>106</sup> Serial isotope scans and angiographic studies of the liver may be of help in the diagnosis of HVT<sup>106</sup>; enzymes such as LDH and SGPT usually become elevated, but other tests of liver function appear to be of limited value.<sup>106</sup>

Severe and refractory *headaches* (see above) may be due to small-vessel thromboses or they may be premonitory signs of progressive cerebrovascular thrombosis.<sup>106</sup> Isotopic brain scans and electroencephalograms, however, are usually of little help in monitoring PNH patients with headaches.<sup>106</sup>

The liberation of thromboplastic material from hemolyzed red cells may be responsible for the increased tendency to thrombosis that characterizes PNH.<sup>88,100,106</sup> Others have postulated that intravascular coagulation may be triggered by the interaction of complement components with complement-sensitive PNH platelets,<sup>106</sup> or that vascular occlusion may be caused by stroma derived from broken-down red cells.<sup>106</sup>

*Infections* occur frequently and may be partially attributable to leukopenia or, perhaps, to functional defects of leukocytes. The latter include defective granulocyte migration, decreased stickiness to filters, defective phagocytosis, and increased lysis in acidified serum and in the presence of antibodies.<sup>35</sup> In one series of 53 deaths, five were attributed to infection,<sup>13</sup> but even mild infections may constitute a serious hazard, since they may

precipitate an exacerbation of the hemolytic process,<sup>30,81</sup> or may lead to aplastic or aregenerative crises, which carry the same serious prognosis as in other hemolytic anemias.<sup>102</sup> Occasionally PNH has terminated in *acute myeloblastic leukemia*<sup>61,65,71,123</sup> and has been reported to occur in association with myelofibrosis<sup>45,71</sup> (Chapter 57) or erythroleukemia<sup>10</sup> (Chapter 47).

*Physical examination* may reveal background pallor and a superimposed jaundice or bronze discoloration of the skin. Moderate splenomegaly is usual, and mild to moderate hepatomegaly is sometimes found. The association of splenomegaly with aplastic anemia (see below) is often a clue to the real nature of the disease. Physical examination is otherwise unrevealing.

### Laboratory Findings

**BLOOD.** In most patients *anemia* is severe, the hemoglobin often being less than 6 g/dl. The red cells are usually macrocytic, but there may be considerable variation in their size.<sup>112</sup> Occasionally, when urinary iron loss has been considerable (see below), the red cells may appear hypochromic and microcytic.<sup>39,111,119</sup> Spherocytes and other abnormal red cell shapes are not seen, but occasional red cell fragments have been described.<sup>102</sup> When present, they may indicate a complicating intravascular thrombosis. In addition to polychromatophilia, *normoblasts may be found. There may be a marked relative reticulocytosis*, but the absolute reticulocyte count is often inappropriately low in relation to the severity of the anemia. The osmotic and mechanical fragility of the erythrocytes is normal,<sup>18</sup> and the reaction to the direct Coombs' test is negative.<sup>21</sup>

*Leukopenia* is often detected and may be marked. There may or may not be neutropenia and relative lymphocytosis.<sup>49</sup> The neutrophil alkaline phosphatase often is very low or absent<sup>77</sup> and the leukocyte acetylcholinesterase (AChE) also has been found to be low.<sup>113</sup> Functional leukocyte defects have been demonstrated<sup>75</sup> (see above). *Thrombocytopenia* of moderate degree is common, but

platelet life span and function generally are normal,<sup>33</sup> although membrane defects leading to increased susceptibility of platelets to destruction by complement components have been described.<sup>2,35</sup>

**PLASMA.** The plasma may be golden brown, reflecting the presence of increased levels of unconjugated bilirubin, hemoglobin, and methemalbumin. Predictably, serum haptoglobins are very low and lactate dehydrogenase levels may be very high during periods of active hemolysis.<sup>47</sup>

**URINE.** When there is increased blood destruction, the urine contains increased amounts of urobilinogen. In addition, intravascular hemolysis leads to depletion of serum haptoglobin and this results in the continuous presence of hemoglobin in the glomerular filtrate in the kidney (Chapter 5, page 207; Chapter 20, page 729). Much of the hemoglobin is reabsorbed by the cells of the proximal convoluted tubules, which become heavily laden with iron. The excretion of this iron in the form of granules gives rise to hemosiderinuria.<sup>53</sup> The urinary sediment is tobacco-yellow in color and gives a positive iron reaction in the Prussian blue stain. Brown granules of altered blood pigment may be seen in the leukocytes or epithelial cells, or outside the cells. In addition, spectroscopic examination may reveal the presence of varying amounts of free hemoglobin.

The *hemosiderin* can be demonstrated as follows: 15 ml of urine are centrifuged in a graduated 15-ml centrifuge tube. The supernatant solution is drawn off down to the 1-ml mark and discarded. The sediment is suspended in the supernatant solution and to this is added an equal volume of 5% hydrochloric acid. After mixing, 0.5 ml of a 10% aqueous solution of potassium ferrocyanide is added. This is mixed and a drop is examined microscopically. When the reaction is positive, blue granules of hemosiderin will be seen, especially within the cells.

A permanent preparation can be made by smearing the urinary deposit on a glass slide and allowing this to dry in air, after which it is fixed by dipping the slide in methyl

alcohol for 10 to 20 minutes. It is then stained for 10 minutes in freshly prepared acid-potassium ferrocyanide solution that is kept at 56° C in a water bath. The slide is washed in running water for 20 minutes, rinsed in distilled water, and finally counterstained with 0.1% safranin or eosin.

The continuous loss of relatively large amounts of iron in the urine may produce *iron deficiency*.<sup>135</sup> Average daily excretions of up to 15.9 mg have been observed<sup>21</sup> and as much as 3.6 mg in 24 hours have been demonstrated even in the absence of hemoglobinuria.<sup>8</sup> It is noteworthy that, despite the continuous presence of hemoglobinuria, *renal function* usually is not seriously affected<sup>120</sup> unless there is some unrelated complicating factor. Albumin has been detected immediately before and after an episode of hemoglobinuria,<sup>14</sup> but usually no protein can be demonstrated between attacks.

**BONE MARROW.** Normoblastic hyperplasia is the characteristic finding. As many as 50% of the nucleated cells may be normoblasts<sup>122</sup>; only occasionally are megaloblastic changes seen.<sup>135</sup> The number of megakaryocytes may be decreased. When there is pancytopenia, a hypoplastic marrow may be found, although in many patients pancytopenia is associated with a cellular marrow.<sup>85</sup>

**SPECIFIC SEROLOGIC TESTS.** The diagnosis of PNH is based on a series of special tests that exploit the sensitivity of PNH red cells to lysis by small amounts of complement.<sup>63</sup>

The susceptibility of PNH erythrocytes to lysis in acidified human serum forms the basis of the *Ham test*.<sup>40</sup> The optimum pH for this reaction is between 6.5 and 7.0. The patient's serum should not be used for the test since it may have been depleted of complement and other heat-labile serum factors.<sup>5</sup> Instead, ABO compatible normal serum, obtained by defibrination of the blood in an open flask, preferably from a donor known to have potent serum,<sup>127</sup> is used. The serum is acidified by adding one volume of 0.2N HCl to nine volumes of serum.<sup>19</sup> Nine volumes of acidified serum are in turn added to one volume of a 50% suspension of washed red corpuscles

obtained from the patient. Frank hemolysis is seen with PNH erythrocytes but may also occur with those from patients suffering from hereditary dyserythropoietic anemia (HEMPAS, page 700) or from spherocytosis, either hereditary or acquired, since spherocytes are easily hemolyzed at hydrogen ion concentrations that leave normal cells intact. The hemolytic property of HEMPAS cells differs from that of PNH cells in that not all normal sera produce acid hemolysis of HEMPAS cells, hemolysis does not occur in the patient's own serum, and reaction to the sucrose hemolysis test (see below) is negative.<sup>63</sup> In order to exclude the possibility of acid hemolysis due to spherocytosis or antibodies, two controls are included in the test system<sup>20</sup>: (1) patient's corpuscles suspended in acidified heat-inactivated serum; and (2) normal corpuscles suspended in the patient's acidified serum. Both will yield negative results in patients with PNH. A third control test may be made by adding the patient's corpuscles to normal serum. Little or no hemolysis will take place if the patient has PNH, whereas hemolysis may occur if immune hemolysins are present. In the latter situation the reaction to Coombs' test, which is negative in PNH patients, will also be positive. Heat inactivation does not, of course, prevent acid hemolysis of spherocytes.

The *thrombin (Crosby) test* is simpler but probably less specific than the Ham test.<sup>20,59</sup> It depends on the increase in hemolysis that occurs when bovine thrombin is added to an acidified cell-serum suspension.<sup>16</sup> Hemolysis is attributed to the presence of heterophil hemolytic antibody contaminating the bovine thrombin preparations, but some enhancement may also occur as the result of other contaminating proteins such as complement components.<sup>63</sup>

The excessive hemolysis of PNH erythrocytes in solutions of low ionic strength containing small amounts of normal serum forms the basis of the so-called *sugar water test*<sup>51</sup> and the *sucrose water test*.<sup>44,54,64</sup> Isotonic solutions of other simple sugars or sugar alcohols will have the same effect, provided the compound selected does not permeate the red

cells.<sup>66</sup> Since all red cells become coated with complement components during incubation in these solutions, it is thought that the positive reaction ultimately depends on the increased sensitivity of PNH red cells to lysis by complement. In these solutions, complement appears to be activated by antigen-antibody-like reactions<sup>51</sup> or in the alternate pathway C3A activator system<sup>37</sup> (see also page 325). A *screening test*<sup>54</sup> consists of adding one part of citrated or oxalated blood to nine parts of freshly prepared sugar water (10 ml of commercial sugar dissolved in distilled water to a final volume of 100 ml). The red cell suspension is gently mixed and incubated for 30 minutes at room temperature. The red cells are then sedimented by centrifugation and the supernate is inspected for gross hemolysis. If no hemolysis is present, the patient probably does not have PNH, provided he has not had recent massive transfusions. A more refined and confirmatory sucrose hemolysis test has been detailed elsewhere.<sup>54,63</sup> It should be stressed that heparin or EDTA are unsuitable as anticoagulants for this test; they frequently lead to false negative results. Defibrinated blood should also not be used, since false positive hemolysis may occur in disorders other than PNH.<sup>54</sup>

The *heat test* is a useful and simple screening procedure.<sup>56</sup> Two to 3 ml of blood are clotted in a glass test tube and allowed to incubate at 37° C from one to three hours. The clot is then removed, and the remaining contents of the test tube are centrifuged. In PNH, the supernatant serum is bright red. The test also yields a positive result in the presence of spherocytes and certain anti-red cell antibodies, but a negative result is strong evidence against the diagnosis of PNH.<sup>63</sup>

*Hemolytic antibody tests* are based on the observation that PNH cells are lysed by smaller quantities of anti-red cell antibodies than are normal cells.<sup>21,117</sup> These tests generally utilize the cold agglutinin, anti-I, and the need for such specific antibodies constitutes the major disadvantage of the test. In addition, the sensitivity of the system has to be adjusted in such a way that PNH erythrocytes are hemolysed, while other cells are not.<sup>63</sup>



## Differential Diagnosis

The diagnosis of PNH must be considered in any patient who has (1) signs of intravascular hemolysis of undefined cause, especially in the presence of hemoglobinuria; (2) pancytopenia in association with hemolysis, whether the marrow is cellular or not; (3) persistent, poorly explained iron deficiency, especially when accompanied by hemolysis; (4) evidence of recurrent venous thrombosis, especially intra-abdominally; and (5) unexplained recurrent bouts of abdominal pain, low backache, or headache in the presence of chronic hemolysis. PNH must be differentiated from antibody-mediated hemolytic anemias, especially paroxysmal cold hemoglobinuria (page 925) and the cold agglutinin syndrome (page 921), and from HEMPAS (page 700).

## Treatment

No definitive therapy is available and management is complicated by a highly variable clinical picture. Some patients have only a moderate degree of anemia, with little hemoglobinuria, and in such patients "he prescribes best who prescribes least."<sup>13</sup> Others have severe anemia punctuated by hemolytic crises, thromboses, and infection; in such patients treatment is necessary but is often unsatisfactory.

*Blood transfusions are valuable in the therapy of anemia, but must be given in the form of saline-washed red cells<sup>18</sup> since fresh donor plasma may accelerate the hemolytic process by supplying labile factors essential for hemolysis. Presumably these factors are consumptively depleted in individuals suffering from chronic hemolysis. Others have attributed febrile and even hemolytic transfusion reactions to the presence of leukoagglutinins<sup>52</sup> and have used leuko-filtered blood in some patients. Normal cells survive well in patients with PNH, and transfusion to nearly normal hemoglobin levels has been observed to produce short-lived remissions.<sup>18,49</sup> This may be due to a temporary decrease in the production of abnormal cells, with a consequent reduction of hemolytic and other phenomena.*

Sooner or later iron deficiency develops in most patients<sup>52,135</sup>, sometimes even though repeated transfusions have been administered. The iron loss must be replaced. Most patients tolerate oral iron therapy well,<sup>50</sup> but accelerated disease activity has been observed in many,<sup>53</sup> and has also been reported following administration of iron-dextran.<sup>60</sup> This phenomenon has been attributed to cell damage caused by iron-catalyzed peroxidation of erythrocyte lipids<sup>60</sup> but more probably results from the outpouring of young erythrocytes, which may be more "sensitive" than the older antecedent population. It has been suggested that iron can be given without major complications if reticulocytosis is first suppressed by transfusions, but this hypothesis requires confirmation.<sup>62</sup>

*Steroids usually are not helpful<sup>31,69</sup> but may be useful in a few patients,<sup>31,52</sup> some of whom have had a positive reaction to the antiglobulin test,<sup>31</sup> a rare occurrence in PNH. It has been suggested that steroids be tried in patients unresponsive to other measures and in females of childbearing age.<sup>52</sup> High doses (40 mg prednisone daily) should be given for at least two weeks before a patient is considered unresponsive. If the patient does respond, maintenance doses of 5 to 10 mg should be employed.<sup>52</sup>*

*Androgen therapy may possibly be of some benefit.<sup>50,52,53</sup> Fluoxymesterone in large starting doses (60 mg daily) and in maintenance doses of 10 to 30 mg daily has been employed. This may result in elevated hemoglobin levels or decreased transfusion requirements.<sup>52</sup> The improvement appears to be accounted for by decreased hemolysis rather than increased erythropoiesis, even though the basic cellular defect is unchanged.<sup>52</sup>*

*Anticoagulants have been advocated for two purposes: (1) to reduce the amount of hemolysis and (2) to prevent or treat thromboses. Because heparin does block the hemolysis of PNH cells in vitro,<sup>52</sup> some believe that it may also reduce hemoglobinuria when given to patients<sup>71</sup>; others have been unable to demonstrate any benefit,<sup>52,73</sup> and some claim that severe hemolytic reactions*

may even be precipitated by heparin.<sup>13,32</sup> It is likely that hemolysis is not influenced one way or another.<sup>52</sup> The use of coumadin and related compounds has also given varied but generally disappointing results<sup>15,21,52,60,101</sup> and is not currently recommended for the treatment of chronic hemolysis.

Anticoagulation probably does have a place in the treatment and prevention of venous thromboses.<sup>15,52,104</sup> Since thromboses are particularly common following surgical procedures and in the puerperium<sup>52</sup> it has been suggested that, in these situations, patients be anticoagulated at the first sign of intravascular thrombosis. Heparin has also been advocated as the agent of choice in the treatment of the patient having progressive, diffuse hepatic vein thrombosis (HVT). Although it has afforded temporary relief, it has failed to halt the inexorably downhill course.<sup>106</sup> Coumadin has proved equally inadequate in protecting against the recurrence of clinically obvious HVT.<sup>100</sup>

*Dextran* (molecular weight 72,000 or preferably 142,000) has been shown to inhibit PNH hemolytic tests in vitro<sup>21,34,36</sup> and has been used for the temporary control of hemolysis associated with infection, trauma, and transfusion reactions.<sup>31,36,129</sup> But its long-term use is dangerous because hemorrhagic complications, antibody formation, and anaphylactic reactions will likely develop. Generally 0.5 to 1.0 l of a 6% solution is sufficient to decrease temporarily the rate of hemolysis. *Dextran's mode of action* is unknown. Penicillamine,<sup>30</sup> tocopherols, 6-mercaptopurine, and a number of other drugs<sup>52</sup> have been tried without success.

*Splenectomy* has provided no permanent benefit and has been followed by death in some instances.<sup>122</sup> A few patients have been reported to have less frequent episodes of hemoglobinuria after splenectomy than prior to it,<sup>21,40</sup> but this may have been coincidental.

In one patient with PNH and marrow failure, the successful transplantation of marrow from a histocompatible sibling resulted in restoration of marrow cellularity and normal hematopoietic function.<sup>128</sup>

## Course and Prognosis

PNH is a chronic disease, the median survival being approximately 10 years.<sup>25</sup> Some patients have survived for 20 years or longer after diagnosis, and one for 43 years.<sup>11,25</sup> The severity of the disease is reflected in the degree of anemia, which in turn is a function of the proportion of complement-sensitive cells and the degree of marrow aplasia. Complications are less predictable. Thromboembolism, a major cause of death, is particularly associated with increased hemolysis, whereas hemorrhage and infection are the most common causes of death in patients suffering from marrow aplasia.<sup>25</sup>

In some PNH patients the severity of the illness lessens with time and a small number achieve a complete clinical remission.<sup>23</sup> Some patients in clinical remission retain laboratory abnormalities for years,<sup>11</sup> whereas others lose all laboratory signs of PNH.<sup>25</sup>

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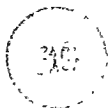
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## Polycythemia

### General Considerations

#### Pathologic Physiology

### Relative Erythrocytosis

#### Galsböck's Syndrome, Stress Erythrocytosis

### Absolute Erythrocytosis

#### Anoxic Erythrocytosis

#### Abnormal Hemoglobins

#### Tumors and Miscellaneous Disorders, Inappropriate Erythrocytosis

#### Benign and Familial Erythrocytosis

### Polycythemia Vera

#### Pathogenesis

#### Symptomatology

#### Diagnosis

#### Treatment

#### Course, Complications, and Prognosis

## General Considerations

### Definitions and Terminology

The term "polycythemia," which means "many cells," is used commonly to refer to an increase in red blood corpuscles, without any implication regarding the number of leukocytes or platelets. However, an increase in the concentration of red corpuscles, whether measured as number of cells, hemoglobin, or packed cell volume, is more correctly designated as *erythrocytosis*. The contrasting condition, *erythremia*, in which the numbers of leukocytes and platelets usually are increased

also, is a disorder of the hemopoietic system. It is more commonly called *polycythemia rubra vera* or *polycythemia vera* (PV).

### Relative and Absolute Erythrocytosis

Erythrocytosis may or may not be associated with an increase in the total quantity of red cells in the body, ie, an increase in red cell mass. *Relative (or pseudo) erythrocytosis* occurs when, through a loss of blood plasma, the concentration of red corpuscles becomes greater than normal; the total number in the circulating blood is not increased. Such events are usually of brief duration (page 975). *Absolute erythrocytosis* refers to a true increase in the total number (mass) of circulating red cells. A rapid, *transient erythrocytosis* may develop in some species of animals when red corpuscles are shunted into the circulation from the splenic storage pool, but this does not occur in normal man (Chapter 8). Absolute erythrocytosis in man is chronic and results from a sustained increase in red cell production. Since red cell survival in erythrocytosis and in polycythemia vera usually is normal, modest increases in erythrocyte production lead to proportionate increases in red cell mass. Furthermore, as red cell mass increases above normal levels, total blood volume also increases (Fig. 30-1), although this occurs somewhat unpredictably because of variable changes in plasma volume.<sup>18</sup>

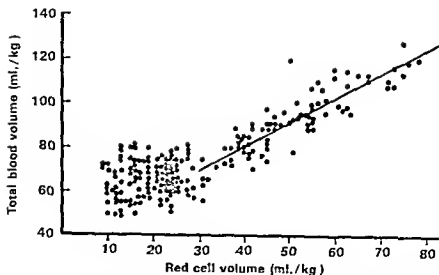


Fig 30-1 The relationship of red cell mass to total blood volume in persons with normal, and increased red cell volume (From Huber et al,<sup>18</sup> courtesy of the author the British Journal of Haematology)

#### Clinical Manifestations and Pathologic Physiology

The clinical manifestations of erythrocytosis are partly related to the underlying disorder. In addition, the increased blood volume and increased blood viscosity that occur in erythrocytosis and in polycythemia vera in themselves produce certain symptoms and signs. These are related to the degree of the increase and the resulting effects on blood flow and oxygen transport. Thus, the "ruddy cyanosis" seen in patients with polycythemia vera is a consequence of the expanded blood volume and resulting dilatation of cutaneous vessels, coupled with the somewhat sluggish flow therein, which, in turn, is caused by the increased blood viscosity.<sup>13</sup> The "black cardiac" (page 980) has a similarly expanded blood volume and vascular system, but, in addition, suffers from a lack of adequate oxygenation of the circulating hemoglobin. Headache, dizziness, tinnitus, a full feeling in the head, and a tendency to bleeding and/or thrombosis may develop in patients with erythrocytosis and an enlarged blood volume regardless of the basic cause.<sup>25</sup> These symptoms usually are relieved by phlebotomy.

#### Blood Viscosity, Blood Volume, and Blood Flow, and Oxygen

It has been difficult to achieve understanding of the clinical of erythrocytosis because of technical limitations. Neither blood volume nor blood viscosity is easily measured (The most accurate measurement of blood viscosity in man were made by allowing blood to flow through an 18-gauge mm diameter) under a mean pressure of 200 to 400 mm saline) and rate.<sup>30</sup> The blood viscosity was calculated on the basis of Poiseuille's law, in part, that the resistance to flow in capillaries is proportional to the length of the capillary (raised to the fourth power), which is valid for Newtonian fluids, but which does not change with time but is not strictly applicable to non-Newtonian fluids such as blood. The resulting data showed that under conditions of study, blood viscosity increased rapidly with increasing packed cell volume (Fig. 30-2). However, the data do not apply to rigid tubes and

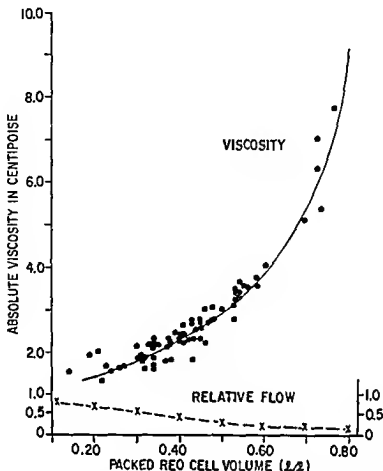


Fig 30-2. The relationship between the absolute viscosity of whole blood and the VPRC is shown in the upper curve (Modified from Pirofsky<sup>30</sup>). In the lower curve, relative flow through a capillary tube of fixed diameter and length and propelled by a fixed pressure gradient is calculated for blood with different VPRCs and thus different viscosity (Modified from Castle and Jandt<sup>10</sup>).

of about 6 ml per minute. At higher flow rates (higher rate of shear) the entire curve would shift downward and to the right (page 124, Fig. 3-25), reflecting a decrease in viscosity.<sup>40</sup> Furthermore, viscosimetry measurements made in capillary tubes yield values that probably are higher than those that occur physiologically.<sup>40</sup> It also is of interest that, at constant values for volume of packed red cells (VPRC), blood viscosity increases as the red cell size decreases. Thus goat and camel erythrocyte suspensions were more viscous than those of humans or dogs.<sup>34</sup>

From blood viscosity values at different

packed cell volumes (Fig. 30-2) it is possible to calculate the rate of blood flow through a tube under a given set of conditions (again somewhat misusing Poiseuille's law).<sup>10</sup> Such calculated values are shown in the lower portion of Figure 30-2 in which it is apparent that, as VPRC and viscosity increase, the calculated flow rate through a capillary of fixed diameter decreases linearly. From these values for blood flow at different values for VPRC and the oxygen content of such bloods, one can calculate the rate of oxygen transport. The result is an inverted arc-like curve as shown in Figure 30-3A which illus-



trates that bloods of different cell content flowing through a capillary tube under a fixed pressure would deliver rather different amounts of oxygen.<sup>10</sup> Very similar curves were obtained for normovolemic dogs (Fig. 30-3B, dashed curve) when the measured

cardiac output and the VPRC values were used to calculate the approximate oxygen transport at different packed red cell volumes.<sup>27,31</sup> In both curves, oxygen transport was low at low VPRC values because of low blood oxygen content (left side of Fig.

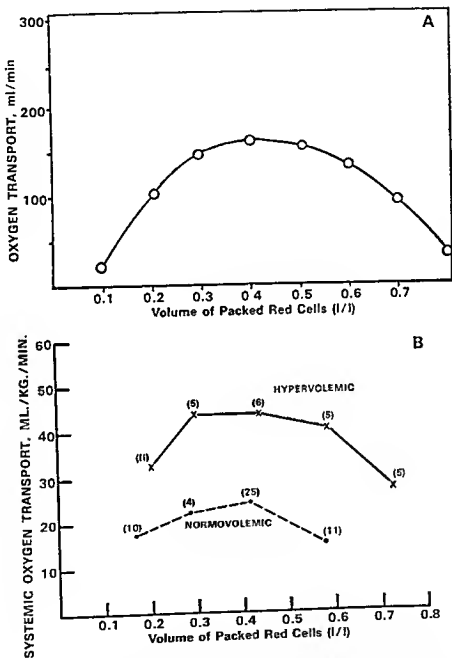


Fig 30-3 Arterial oxygen transport at different VPRCs and thus different viscosity values. A, The values in this curve were calculated from the blood viscosity values as measured by Pirofsky<sup>30</sup> and the flow values calculated in the lower portion of Figure 30-2 B Systemic oxygen transport as calculated from cardiac output measured in normovolemic and hypervolemic dogs (From Murray et al,<sup>27</sup> courtesy of the authors and the *Journal of Clinical Investigation*)

30-3). At high VPRC levels (right side of Fig. 30-3), calculated oxygen transport also is low in spite of high oxygen content because the increase in viscosity results in slowed blood flow. At intermediate levels, calculated oxygen transport is considerably higher and appears to be optimal at about normal hematocrit values.<sup>4,10,27,31</sup> That such an optimum VPRC level for maximal oxygen transport was found in experimental animals with normal blood volumes (Fig. 30-3B) suggests that the calculated values (Fig. 30-3A) may have physiologic significance.<sup>4,27,31</sup> In particular, these observations may explain the effect of anemia in causing tissue hypoxia<sup>5</sup> and increased erythropoietin production.

At elevated VPRC values these same curves predict low oxygen transport and tissue hypoxia, as already mentioned; as a result, increased erythropoietin production would be expected and this should lead to increased numbers of red cells. However, this prediction is not in accord with several observations, namely, the adequate tissue oxygenation<sup>9,36,42</sup> and normal erythropoietin<sup>1,15</sup> values found in hypertransfused animals and in patients with polycythemia vera, as well as the fact that hypertransfusion results in a decrease in erythropoiesis<sup>2,15,42</sup> rather than increasing tissue hypoxia, thereby leading to a vicious cycle of increasing red cell production. The explanation for this apparent paradox may lie in the fact that, as the red cell mass increases, the blood volume does not remain constant; instead, the blood volume expands as the red cell mass expands (Fig. 30-1). One effect of increasing the blood volume is to increase cardiac output, mainly by increasing stroke volume.<sup>12,27</sup> Thus, in acutely hypervolemic dogs, the cardiac output was about twice that of normovolemic dogs with similar VPRC values.<sup>27</sup> Peripheral vascular resistance (PVR) increased also as packed cell volume was increased, but PVR was less in hypervolemic animals than in those with normal blood volumes at similar values for VPRC.<sup>27</sup> The oxygen transport curve in acutely hypervolemic dogs<sup>27</sup> and rodents<sup>38</sup> showed an inverted arc configuration similar to that seen in normovolemic animals

(Fig. 30-3B), but oxygen transport levels were elevated and the maximum value was shifted to the right.<sup>36</sup>

Measurements of cardiac output and calculated oxygen transport in man have yielded somewhat different results.<sup>12</sup> The mean cardiac output in 10 patients with polycythemia vera (mean VPRC 0.60 l/l) was 4.8 l/min/m<sup>2</sup> as compared to 3.65 l/min/m<sup>2</sup> in six normal controls (Fig. 30-4). In contrast, anemic patients showed a marked decrease in oxygen transport in spite of a twofold increase in cardiac output (Fig. 30-4)<sup>8</sup>; in other words, in anemia the increased cardiac output does not fully compensate for the decreased blood oxygen content.<sup>10</sup> When oxygen transport was calculated from these data<sup>10</sup> a divergence from the inverted arc configuration was noted (Fig. 30-4, dashed line). However, it must be noted that in the patients with polycythemia vera the total blood volume presumably was increased (Fig. 30-1), while in the normal and anemic subjects it was not.

The probable situation with respect to oxygen transport in persons with erythrocytosis or polycythemia vera as compared to normal and anemic subjects is illustrated in Figure 30-5. In absolute erythrocytosis and in polycythemia vera, because of the associated hypervolemia, the oxygen transport curve is similar to that in normovolemic states but it is elevated and shifted to the right.<sup>27,36</sup> Thus, in compensated hypoxic states, polycythemia is beneficial because it leads to hypervolemia and increased oxygen transport (compare points 1 and 2 in Fig. 30-5). In patients with absolute erythrocytosis or polycythemia vera, oxygen transport may be increased, normal, or decreased as compared to the normovolemic state (Fig. 30-5, points 3, 4, and 5 respectively), depending on the degree of polycythemia and the resulting changes in viscosity and blood flow.

**RELATION TO TREATMENT OF POLYCYTHEMIA.** The foregoing considerations are of interest in understanding not only the pathophysiology of erythrocytosis and polycythemia vera but also the treatment thereof. In polycythemia vera there is little or no general

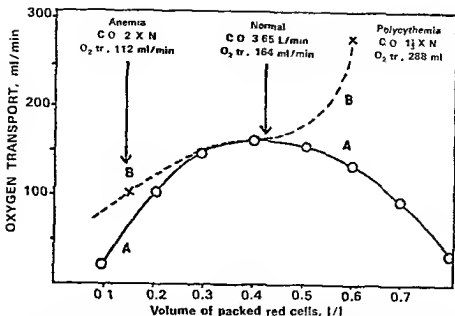


Fig 30-4 Curve A The relative oxygen transport *in vitro* by blood with various values for VPRC (the same curve as in Fig 30-3A) Curve B, Oxygen transport in man derived from measured values obtained in 10 patients with polycythemia vera [cardiac output (CO)  $1\frac{1}{2} \times$  normal] 6 normal subjects [CO 3.65 l/min/m<sup>2</sup>]<sup>12</sup> and in anemic patients [CO  $2 \times$  normal] (From Castle and Jandl<sup>10</sup> courtesy of the authors and Henry M Stratton, Inc.)

need for increased tissue oxygen transport. However, in some local areas where fixed vessel diameter (from arteriosclerosis) limits increased blood flow the additional impeding effect of increased blood viscosity may limit oxygen transport and result in local tissue ischemia; the local vessel changes may interfere with the ability of increased blood volume and resulting increased cardiac output to compensate for the greater viscosity effects at high hematocrit levels. Because of this, when treating by phlebotomy, it is best not to reduce the blood volume too greatly at any one bleeding, especially in patients with known arteriosclerotic symptoms (angina pectoris, transient ischemic attacks, etc.). Rather, time should be allowed for hemodilution to occur between phlebotomies, or in emergencies the blood volume may be maintained by infusing saline or plasma expanders<sup>16</sup> or by reinfusing the removed plasma.<sup>475</sup> Obviously, in such patients a sudden fall in blood volume from other causes, such as dehydration or acute hemorrhage, may also

result in local ischemia because the high viscosity effects cannot be compensated for by increased cardiac output. In patients with congestive heart failure, the need for reduction of blood viscosity may be urgent since the ability to increase cardiac output in order to compensate for the increased blood viscosity has been compromised. The oxygen hemoglobin dissociation curve is shifted to the right in such patients.<sup>22</sup>

In hypoxemic erythrocytosis, blood oxygen transport is less efficient at comparable hemoglobin or VPRC levels than in polycythemia vera because of arterial oxygen unsaturation. Therefore, the curves for oxygen transport would be lower than in situations in which hemoglobin oxygenation is normal (Fig. 30-5). In the presence of decreased arterial oxygen saturation, tissue hypoxia may persist even when there is marked erythrocytosis. The main advantage to be derived from decreasing blood viscosity and blood volume in hypoxic (secondary) erythrocytosis is to decrease the cardiac work

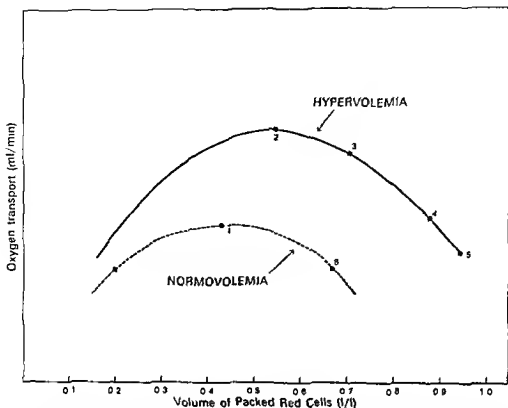


Fig 30-5 Hypothesis depicting the relationship between oxygen transport and VPRC in normovolemic and hypervolemic situations. Cardiac output is assumed to remain constant. Point 1 refers to a normal subject at rest, with normal VPRC and normal oxygen transport. Point 2 describes the patient with erythrocytosis (elevated VPRC, blood volume and oxygen transport) at rest. Points 3, 4 and 5 refer to the effects of increasing VPRC; oxygen transport presumably decreases from optimal values as the VPRC increases, but may exceed values for oxygen transport in normovolemic patients until very high VPRC values are reached. Point 5 indicates the situation in normovolemic polycythemia produced by exchange transfusion, i.e., increased VPRC, decreased oxygen transport. (Modified from Thorling and Erslev<sup>36</sup> courtesy of the authors and Henry M. Stratton, Inc.)

load. From the curves in Figure 30-5, one would predict that in such situations an increase in tissue oxygen transport should result from phlebotomy. However, the variable and unpredictable results of phlebotomy observed in patients with hypoxia-induced erythrocytosis<sup>21</sup> indicate that factors other than blood viscosity and oxygen transport may be involved. Better results have been reported when exchange transfusion with dextran was used to maintain blood volume.<sup>16</sup> In helping to achieve the best balance between increased cardiac work and decreased tissue hypoxia in patients with hypoxemic erythrocytosis the subjective feelings of the patient probably are the best guides.<sup>10</sup>

### Classification<sup>24</sup>

As stated earlier, erythrocytosis must be distinguished from polycythemia vera; furthermore, in patients in whom hematocrit or hemoglobin levels are found to be increased, it is necessary to determine whether the erythrocytosis is relative or absolute. The various forms of absolute erythrocytosis are listed in Table 30-1. These and polycythemia vera can be differentiated from relative erythrocytosis on clinical grounds, as will be described below, and also, when necessary, by measurements of blood volume. The latter, however, are not easily interpreted. Normal values are presented in Table 30-2, but there

**Table 30-1. Classification of Polycythemia**

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I	Erythrocytosis
A.	Relative erythrocytosis (pseudoe erythrocytosis)
1	Hemoconcentration burns, shock, acute ascent to high altitude, diarrhea prolonged sweating
2	Stress erythrocytosis (Gaisböck's syndrome, ?normal variant)
B.	Absolute erythrocytosis
1	Secondary to decreased tissue oxygenation (anoxic erythrocytosis)
a	High altitude
	Monge's disease
b	Pulmonary disease
	Chronic cor pulmonale
	Ayerza's syndrome
c	Congenital heart disease
d	Hypoventilation syndromes
	Primary alveolar hypoventilation
	Pickwickian syndrome Ondine's curse
e	Abnormal hemoglobins
	(1) Inherited
	(2) Acquired, drugs and chemicals
2	Secondary to aberrant erythropoietin production tumors cysts, hemangiomas, etc
3	Benign erythrocytosis
4	Benign familial erythrocytosis
II	Polycythemia vera (erythremia)

---

is considerable variation from one subject to another in red cell, plasma, and total blood volume, even when expressed as ml/kg body weight. This variation results, in part, from differences in body fat content<sup>19,20,28,41</sup>; thus, blood volume is more closely related to lean body mass<sup>28</sup> than to weight or surface area.

It is a common practice to measure either plasma volume or red cell volume, and, from one of these determinations, total blood volume is calculated on the basis of the relative amounts as indicated by a hematocrit determination. In the view of most<sup>6,7,38</sup> but not all<sup>34</sup> investigators, this practice increases the chance of error. It is better to measure red cell mass and plasma volume separately.

It should be emphasized that blood volume measurements do not differentiate between absolute erythrocytosis and polycythemia vera, and thus they are useful only in distin-

guishing absolute from relative erythrocytosis.

The classification presented in Table 30-1 is based on pathophysiologic as well as clinical grounds. It is now recognized that red cell production at the erythroblast level may be stimulated by erythropoietin. Decreased tissue oxygenation, no matter what the cause, leads to increased production of erythropoietin (Chapter 4), but this hormone may also be elaborated "inappropriately," as in certain tumors, renal cysts, and the like. It has also been found that hematopoiesis, beginning at the stem cell level, may increase autonomously, with only partial regulation by the usual homeostatic mechanisms of oxygen tension and erythropoietin; the latter phenomenon occurs in polycythemia vera.<sup>1,32</sup> On theoretical grounds, it might be expected that measurement of serum or urine erythropoietin levels would be useful in differentiating the various forms of polycythemia. This has not yet proved to be the case. First of all, high serum levels of erythropoietin are not usually found in patients who are responding to endogenous erythropoietin; rather, elevated serum levels are found in the sera of patients with faults in erythropoiesis, as in aplastic anemia.<sup>38</sup> Furthermore, values even for urinary excretion of erythropoietin over a few hours<sup>75</sup> have varied widely.<sup>1,2</sup> Most important of all is the fact that methods for measurement of erythropoietin that are both accurate and sensitive, and are also widely available, have not yet been described.

## Relative Erythrocytosis

### Transient Erythrocytosis

Lowered fluid intake, marked loss of body fluids, or a combination of both will cause a decrease in plasma volume and relative erythrocytosis. This decrease in plasma volume occurs when there is persistent vomiting, severe diarrhea, especially with copious sweating, and postoperatively,<sup>57,66</sup> and also soon after an individual reaches a high altitude.<sup>61</sup> A less obvious cause is an increase in

**Table 30-2. Normal Values for Red Blood Cell, Plasma, and Total Blood Volume (ml/kg  $\pm$  1 SD)\***

	Number	Red Blood Cells	Plasma	Total Blood Volume
<b>WOMEN</b>				
<i>Sea level</i>				
Wennesland et al <sup>41</sup>	97	25.4 $\pm$ 2.6	36.8 $\pm$ 3.7	—
Huff and Feller <sup>19</sup>	20	24.4 $\pm$ 2.6	34.8 $\pm$ 3.2	58.9 $\pm$ 4.9
<b>MEN</b>				
<i>Sea level</i>				
Wennesland et al <sup>41</sup>	199	28.3 $\pm$ 2.8	34.4 $\pm$ 4.0	—
Huff and Feller <sup>19</sup>	42	28.3 $\pm$ 4.1	33.5 $\pm$ 5.2	61.5 $\pm$ 8.6
Weil et al <sup>39</sup>	16	27.1 $\pm$ 3.7	33.0 $\pm$ 5.3	60.0 $\pm$ 8.6
<i>1600 Meters†</i>				
Weil et al <sup>39</sup>	19	26.8 $\pm$ 3.2	31.9 $\pm$ 3.8	58.7 $\pm$ 5.8
<i>3100 Meters†</i>				
Weil et al <sup>39</sup>	39	31.8 $\pm$ 6.7	35.2 $\pm$ 5.3	66.8 $\pm$ 8.5

\*RBC volume measured by <sup>51</sup>Cr method. Other values calculated without correction for trapped plasma.

†These are the only values of which we are aware at altitudes significantly above sea level. They may be somewhat low for unknown reasons since the packed cell volumes at 1600 meters were the same as at sea level, a finding in contradiction with our own large experience.

insensible fluid loss, as occurs in fever, hyperthyroidism, or diabetic acidosis. By other mechanisms, loss of electrolytes from the extracellular compartment without concomitant loss of body water leads to a decline in osmolar concentration in the extracellular fluid. The resulting shift of water into the tissue cells may produce relative polycythemia, sometimes of high grade.<sup>57</sup> In burns, loss of plasma leads to hemoconcentration. Also, in certain types of circulatory failure there may be a loss of plasma into the interstitial spaces; such a shift takes place largely in the capillary beds with the result that erythrocytosis may be more marked there than in the central blood vessels.<sup>57</sup> In most of these situations the cause of the hemoconcentration is apparent from the history and examination of the patient, and the process is of brief duration, lasting only a few hours to several days.

#### Chronic Forms

These have been variously referred to as Gaisbock's syndrome,<sup>53</sup> "stress" erythrocyto-

sis,<sup>60</sup> benign polycythemia,<sup>64</sup> benign erythrocytosis,<sup>64</sup> spurious polycythemia,<sup>50</sup> or pseudopolycythemia.<sup>58</sup> In one large series of 215 patients referred with a diagnosis of polycythemia vera,<sup>62</sup> 18 were thought to have chronic relative erythrocytosis, and it was postulated that this might be caused by stress.<sup>60</sup> These patients were predominantly males with an average age of 43 years (16 to 68), and thus many were younger than patients who have polycythemia vera. Many were mildly obese; also many had hypertension. In 6 of the patients the volume of packed red cells was in the high normal range, while in 12 the values were greater than 0.50 l/l. The leukocyte counts were normal ( $< 11.0 \times 10^9/l$ ). Although 11 had "ruddy cyanosis," none had a palpable spleen. The red cell volume was normal, but the plasma volume was subnormal. Follow-up for 6 to 24 months, during which time no treatment was given, revealed no change in clinical symptoms or laboratory findings.<sup>60</sup> Similar groups of patients have been described in other reports.<sup>54,58,66</sup> In one study<sup>66</sup> the VPRC was found to be greater than three

standard deviations above the normal mean in 18 of 25 patients, ranging up to 0.65 1/1. However, total red cell volume, determined in 10 of the subjects, was increased in only 3 of this group. In retrospect, perhaps the 3 patients suffered from true erythrocytosis secondary to some obscure cause such as an abnormal hemoglobin (page 982); the other 7 apparently had relative erythrocytosis.

It is doubted that stress erythrocytosis and Gaisböck's syndrome are true clinical entities.<sup>50,52</sup> The view that an arbitrary value for volume of packed red cells should be considered as the upper limits of normal has no statistical foundation. The values generally accepted as normal at sea level, or at any given altitude, represent the mean  $\pm 2$  standard deviations. This indicates that, on the basis of the normal frequency distribution curve for this physiologic parameter, the values in 2.3% of the population lie above this range. Such "odd men out"<sup>52</sup> should not be regarded as necessarily being abnormal. Also the evidence that hypertension or stress is a pathogenetic factor is tenuous.<sup>52</sup> Attempts to produce stress erythrocytosis in rats were unsuccessful.<sup>56</sup>

Many individuals with relative erythrocytosis unfortunately have had venesections and have even been treated with radioactive phosphorus. That phlebotomy is neither called for nor justified is indicated by the fact that it has not proved useful in alleviating symptoms such as dizziness, in contrast to the relief of these symptoms in polycythemia vera afforded by this means.<sup>62,66</sup> The differential diagnosis of these conditions and polycythemia vera is discussed on page 995.

## Absolute Erythrocytosis

An absolute increase in red cell mass may result from a variety of causes but also is the chief manifestation of the hemopoietic disorder, polycythemia vera.

### Anoxic Erythrocytosis

Insufficient oxygen supply to the tissues may come about as a result of: (1) decreased

atmospheric oxygen pressure, eg, high altitude; (2) pulmonary diffusion or mixing abnormalities; (3) right-to-left cardiopulmonary shunts as in congenital heart disease; (4) hypoventilation; or (5) impaired oxygen-carrying capacity of hemoglobin. In all of these disorders the deficient supply of oxygen in the tissues is presumably detected by some sensing organ(s), possibly in the kidney, and this in turn leads to the production of erythropoietin and an increase in red cell mass (page 180, Chapter 4).

### At High Altitudes

In 1890, Viault<sup>95</sup> showed that erythrocytosis develops during sojourn at high altitude. He found erythrocyte counts of  $7.5$  to  $8.0 \times 10^{12}$  cells/l not only in natives living in the Peruvian Andes and working in a mine at an altitude of 4,392 m above sea level, but in himself and in a fellow traveler as well, although his blood count in Lima (160 m) had been normal. In a Himalayan expedition it was demonstrated that red cell volume and total hemoglobin rose progressively as higher altitudes were attained and at 19,000 feet (5800 m) reached mean values 49% above those at sea level. The increase in total blood volume was partially masked by reductions in plasma volume.<sup>89</sup> Similar responses to rarified atmospheres have been observed in animals.<sup>98</sup>

The rapid ascent to high altitude is accompanied by symptoms of fatigue, dizziness, pulsating headache, anorexia, nausea, vomiting, insomnia, and irritability, a syndrome well known to mountain climbers and residents of high altitudes and referred to as acute mountain sickness.<sup>78,84,92</sup> The symptoms first appear some 4 to 6 hours after reaching a high altitude, but may be delayed for up to 96 hours, suggesting that the pathogenesis may be more complex than simple hypoxia. The incidence and severity of these symptoms are related to the altitude achieved and the susceptibility of the individual. Thus, all persons will develop symptoms if suddenly transported from sea level to 15,000 feet (4,570 m) or higher, while a few develop

symptoms at 8 to 10 thousand feet (2400 to 3000 m).<sup>78</sup> After 4 to 8 days, acclimatization usually occurs and symptoms remit spontaneously.<sup>92</sup> In some individuals, however, symptoms may progress to cerebral confusion, coma, and even death due to pulmonary edema<sup>92</sup> unless the subject is returned to low altitude.<sup>77</sup> The pathogenesis of acute mountain sickness is thought to involve hypoxia and subsequent excessive secretion of anti-diuretic hormone and adrenal steroids with resulting fluid retention, increased blood volume, and finally cerebral edema and/or pulmonary congestion.<sup>92</sup> The incidence and severity of symptoms can be considerably reduced by administering diuretics such as acetazolamide or furosemide.<sup>90,92</sup>

The events that are associated with acclimatization after arrival at high altitude are not understood completely but probably include: (1) An increase in erythrocyte 2,3-DPG levels<sup>83</sup> and a shift to the right in the oxygen-hemoglobin dissociation curve,<sup>77,82,87</sup> thus allowing better tissue delivery of oxygen in spite of decreased arterial oxygen saturation (Fig. 3-16). The increase in 2,3-DPG appears to more than compensate for the left shift in the curve that results from the initial hypocapnia and increase in arterial pH.<sup>77,92</sup> (2) An increase in plasma and urinary erythropoietin<sup>96</sup> levels with subsequent increase in plasma iron turnover, reticulocytosis,<sup>75</sup> and increase in red cell mass and blood volume. (3) Subsidence of the initial excessive anti-diuretic hormone and adrenal steroid secretion and return to the normal diurnal variation of plasma steroid levels.<sup>92</sup> The final result is a new equilibrium at decreased oxygen saturation and carbon dioxide tension with increases in alveolar ventilation, respiratory frequency, and red cell mass.<sup>77,78</sup> These manifestations of acclimatization are quickly lost on descent to sea level even after many years of residence at high altitude.

In some well-acclimated individuals, after a few or many years of good adaptation to high altitude, excessive erythrocytosis (beyond the expected altitude response) is noted. This progresses to hematocrit values approaching 0.80 l/l, and often an incapac-

itating illness characterized by alveolar hypoventilation develops. This entity is known as *chronic mountain sickness* or *Monge's disease*.<sup>78,85</sup>

Monge<sup>84</sup> described an "emphysematous type" and an "erythremic type." In the *emphysematous* form the dominant symptom is dyspnea and there is a long history of bronchitis and laryngitis. Cyanosis is present. The thorax is globular and the vital capacity is diminished. The *erythremic* type, when mild, is characterized by some diminution in mental and physical fitness, fatigue, an erythremic color that turns to cyanosis on the least exertion, occipital headache, anorexia that increases as the day goes on, nausea, vomiting, diminution of visual acuity, and paresthesias. In other instances, headache, dizziness, tinnitus, vague pains in the extremities, cough, and hemoptysis occur. When the disorder is more severe, there is incessant dyspnea, aphroia is common, and there is profound lethargy and even coma. Crises of mental confusion have been observed. Sexual coldness is common. Not only may paresthesias be present but also excruciating pain may be felt in the lower extremities. The face is bluish-violet, the eyelids are edematous and bluish, the sclerae are intensely colored by distended capillaries, the tongue is thick, the hands are enlarged and turgid, and the fingers are clubbed. Hypotension is often present. The liver and spleen have been found to be enlarged only in about 10% of the subjects.

Erythrocytosis is characteristic and is more marked than in other residents of the same altitude who have no symptoms. Counts of  $7.0 \times 10^{12}/l$  are common and  $9.06 \times 10^{12}$  cells/l were found in one of Monge's patients.<sup>84</sup> There is a corresponding, sometimes a slightly greater, rise in hemoglobin and volume of packed red cells.<sup>94</sup> MCV is normal or slightly increased and MCHC is normal.<sup>80,94</sup> Reticulocytes may be increased,<sup>80,84</sup> although in most instances they are normal. The leukocyte count often is normal,<sup>80</sup> although Monge described slight leukocytosis together with monocytosis. Hyperbilirubinemia may be pronounced, and is due mainly to unconjugated bilirubin. There is greater



blood destruction, as measured by fecal urobilinogen, than in normal residents of high altitudes.<sup>83</sup> Remarkable increases in blood volume have been recorded, as great as 212 ml/kg body weight, with 173 ml for red cell volume and 28 to 43 ml/kg for plasma volume.<sup>80</sup> More recent, and probably more accurate, determinations have indicated a less striking although still greatly increased red cell volume (88 to 95 ml/kg body weight).<sup>83</sup> Although platelet counts have been found to be normal or high, clot retraction is poor. Epistaxis is common and hemoptysis, bleeding of the gums, and purpura may occur.

Those affected usually are in the fourth to sixth decade of life. Remissions and relapses are described. Ascent to still higher altitudes results in aggravation of the symptoms, whereas descent to sea level relieves them. Cardiac impairment does not appear until late and death has occurred more often from hemorrhage, pulmonary tuberculosis, or bronchopneumonia than from cardiac insufficiency.

The decreased oxygen saturation of the arterial blood in individuals residing at high altitudes is not correlated with the development of symptoms of chronic mountain sickness. In natives with chronic mountain sickness the arterial  $p\text{CO}_2$  is higher than in their own native control group.<sup>85</sup> In residents of high altitudes it appears that the several forms of chronic mountain sickness reflect the development of one or another of the types of chronic lung disease also seen in persons residing at sea level. It is plausible to assume that intrinsic pulmonary disease, too mild to cause signs or symptoms at sea level, is the cause of Monge's disease in many instances. This was the finding in a patient observed in the Rocky Mountains of the United States.<sup>79</sup> The erythremic type may be due to inappropriately low ventilatory response, secondary to reduced respiratory sensitivity to  $\text{CO}_2$ ,<sup>85</sup> and thus may be similar to some hypoventilation syndromes seen at sea level (page 981). There is, in fact, considerable clinical similarity between Ayerza's disease (page 980) and chronic mountain sickness. A person afflicted with the former

disease, however, could probably not live at altitudes of 10,000 feet (3000 m) or higher.

Differential diagnosis should not be difficult. Cases of congenital heart disease can be distinguished by the cardiac findings. In acquired heart disease, cyanosis is associated with signs of cardiac failure, whereas such signs are lacking when cyanosis is present in Monge's disease. The chief distinction from polycythemia vera is the failure of polycythemia to be altered by increased oxygen pressure. In Monge's disease, descent to sea level brings about complete relief of symptoms, together with a pronounced reduction in the blood volume and restoration of normal blood counts.<sup>80</sup>

### Pulmonary Disease

A variety of diseases, such as chronic obstructive lung disease, diffuse pulmonary infiltrates (fibrous or granulomatous), kyphoscoliosis, and multiple pulmonary emboli, lead to erythrocytosis as the result of inadequate oxygenation of the blood circulating through the lungs. However, not all patients with lung disease and decreased arterial oxygen saturation have elevated hemoglobin or hematocrit levels<sup>118,120</sup> and only in about 50% is there an increase in red cell volume.<sup>127</sup> The reason for this suboptimal response to tissue anoxia is not clear, but it does not appear to result from a decrease in erythropoietin production or the presence of chronic infection.<sup>118,120,127</sup> When erythrocytosis occurs it is usually associated with an increased MCV, reduced MCHC,<sup>130</sup> and a normal MCH.<sup>118</sup> Although it has been suggested that iron deficiency may be a factor in these changes in corpuscular indices,<sup>112</sup> there is little evidence to support this. More plausibly the red cell changes have been attributed to swelling, which in turn may be due to carbon dioxide retention.<sup>118</sup> It has been suggested that carbon dioxide retention may inhibit the marrow response, but no evidence is available to support this.<sup>118</sup> If erythrocytosis is present it can be reduced by continuous oxygen administration.<sup>111</sup>

*Cavernous hemangiomas of the lung may*

be associated with erythrocytosis.<sup>132</sup> *Pulmonary arteriovenous fistula* should be suspected when a peculiar murmur in a lung field together with abnormal roentgenographic shadows (Fig. 30-6) are associated with erythrocytosis, cyanosis, and other symptoms suggesting pulmonary disorder, such as hemoptysis and clubbing of the fingers.<sup>124,126,133</sup>

#### *Chronic Cor Pulmonale*<sup>114</sup>

The clinical picture of this syndrome varies, but oxygen deficiency with arterial desaturation and elevated pulmonary artery pressure are of central importance.<sup>114,122</sup> The erythrocytosis with its associated increase in blood viscosity, increased blood volume, and tendency to thrombosis appears to be the physiologic price of a compensatory mechanism progressively stretched beyond its ameliorating capabilities to the point at which it is more injurious than beneficial.<sup>115</sup> As

already mentioned, phlebotomy may at times be helpful in relieving symptoms, but this is unpredictable (page 974). As in less severe pulmonary disease the MCV of the red cells tends to be increased above normal,<sup>110</sup> whereas MCHC generally is lower than normal.<sup>121</sup>

#### *Ayerza's Syndrome*

This syndrome, characterized clinically by slowly developing asthma, bronchitis, dyspnea, and cyanosis with associated polycythemia, was described by Ayerza in 1901. Congestive failure may be present and there is usually evidence of dilatation and hypertrophy of the right ventricle. Such patients have been referred to as black cardiacs (*cardiacos negros*).<sup>125,129</sup> The essential pathologic change is primary disease of the pulmonary artery or its branches.<sup>131</sup> This has been attributed to syphilis,<sup>133</sup> but more often there is pulmonary arterial and arteriolar sclerosis.<sup>134</sup>



Fig 30-6 Pulmonary arteriovenous fistula associated with polycythemia

In some cases, congenital narrowing or hypoplasia of the pulmonary artery may play a role. The spleen is sometimes enlarged, clubbing of the fingers is present, and chronic heart failure and passive congestion of the liver eventually occur.

### Congenital Heart Disease

Red cell counts of  $7.0$  to  $8.5 \times 10^{12}/l$  are common in persons with congenital heart disease and there are reports of counts as high as  $10.0$  and  $13.9 \times 10^{12}/l$ .<sup>145</sup> The volume of packed red cells was as high as  $0.86$  l/l blood in one of our patients. Erythrocytosis occurs in patients in whom there is a partial shunt of the blood from the pulmonary circuit. The most common defects producing such erythrocytosis are pulmonary stenosis (usually with defective ventricular or atrial septum, patent forameo ovale, or patent ductus arteriosus), persistent truncus arteriosus, complete transposition of the great vessels, and the tetralogy of Fallot (pulmonary stenosis, defective ventricular septum, dextroposition of the aorta, right ventricular hypertrophy). Individuals with such defects exhibit evidence of disturbed cardiorespiratory function, marked cyanosis, clubbing of the fingers and toes, and sometimes stunted growth. It has been reported that an enlarged spleen is not uncommon, but in our experience we have not found this to be the case.

The total plasma volume may be reduced below normal, but the increase in the size of the red cell mass is so great that the total blood volume usually is higher than normal.<sup>141,146</sup> The fatty tissue in the bone marrow is replaced by hematopoietic marrow.<sup>145,148,149</sup>

In a series of 41 subjects<sup>144</sup> the serum bilirubin and the serum iron were found to be increased above normal, but no higher than would be expected from the total increase in hemoglobin metabolism as represented by the erythrocytosis.

It is generally assumed that the underlying stimulus to the hematopoietic system is low oxygen tension resulting from shunting of unoxygenated blood through or around the

lungs with consequent unsaturation of the arterial blood. The arterial oxygen saturation often is as low as 30 to 35%. With successful operative intervention this may rise to 75 and even 88% and, with this, the erythrocytosis disappears. The effect of the anoxia, however, is not a direct one on the bone marrow but is mediated by humoral factors concerned in the regulation of bone marrow activity, as discussed in Chapter 4 (page 180).

### Acquired Heart Disease

In acquired heart disease such erythrocytosis as may develop is of minor degree and is correlated to some extent with the degree of decompensation. The erythrocytosis has been reported as being accompanied by evidence of intensified erythropoiesis in the bone marrow, an increase in red cell mass, and some macrocytosis.<sup>140</sup>

### Hypoventilation Syndromes

Erythrocytosis is found occasionally in patients who exhibit no evidence of pulmonary disease or cardiovascular shunts. Such patients may erroneously be considered as having Gaisbock's syndrome (page 976).<sup>169</sup> The primary defect in at least some of them appears to be an inadequate ventilatory drive from the respiratory center in the brain.<sup>114,163</sup> Such a defect has been found in patients suffering from the *Pickwickian syndrome*, so-called because of the clinical picture of the fat boy described in Dickens' *Pickwick Papers*.<sup>160</sup> In association with extreme obesity, these patients exhibit somnolence, cyanosis, and hypercapnia and may develop periodic respiration, ultimately with right ventricular failure. Voluntary hyperventilation alleviates the hypercapnia, and in many but not all patients, loss of weight restores alveolar ventilation to normal and reverses the syndrome.<sup>113</sup> However, not all massively obese individuals develop alveolar hypoventilation and erythrocytosis. It appears that it is only in the presence of an insensitive respiratory center that a massive panniculus limits respiratory function and results in alveolar

hypoventilation, hypoxemia, and hypercapnia.<sup>161</sup> In some patients the decreased ventilatory drive is of unknown cause,<sup>167</sup> perhaps congenital, or due to idiopathic disease of the medullary respiratory center ("Ondine's curse"<sup>163</sup>), whereas in others it may have resulted from bulbar poliomyelitis, vascular thrombosis, or previous encephalitis.<sup>114,161</sup> In any case, the consequent hypoxemia results in elevated levels of erythropoietin and erythrocytosis, with packed cell volumes as high as 0.75 l/l.<sup>163</sup>

### Abnormal Hemoglobins<sup>220</sup>

Hereditary abnormalities in the hemoglobin molecule and possibly acquired changes in the oxygen-binding capabilities of hemoglobin are among the rarer causes of erythrocytosis.

#### *Hereditary Abnormalities of Hemoglobin*

At least 23 genetic abnormalities in the amino acid sequence of the hemoglobin molecule that result in increased affinity of the hemoglobin molecule for oxygen have been recognized. Of these, six (hemoglobins Köln, Freiburg, H, G<sub>Georgia</sub>, Rampa, and Tacoma) are in various degrees unstable (Chapter 24), and either normal blood values or anemia has been observed rather than erythrocytosis. Erythrocytosis was not reported in association with hemoglobin Denmark Hill,<sup>219</sup> which affects the same structural site as Hb G<sub>Georgia</sub> and Hb Rampa. In Hb Abruzzo<sup>218</sup> the substitution affects the same site as Hb Little Rock, but the hematologic effects, if any, have not been reported. The remaining 15 (Table 30-3) are associated with mild to moderate erythrocytosis. White blood cell and platelet counts have been normal. It seems likely that, in the past, in some individuals with such hemoglobins the diagnosis of familial polycythemia, benign erythrocytosis, or even Gaisböck's syndrome has been made. With the exception of an apparent increase in stillbirths and abortions in some women with hemoglobin Yakima,<sup>202</sup> no significant untoward effects have been observed

and no treatment is indicated. Abnormal maternal-fetal oxygen exchange could explain the fetal mortality.

Inheritance has been autosomal dominant. In the largest families studied,<sup>182,190</sup> as many as 20 individuals in four generations were affected. Most of the abnormal hemoglobins discovered so far have been demonstrable by starch gel electrophoresis in tris-EDTA-borate buffer at pH 8.6, but hemoglobins Rainier<sup>180</sup> and Bethesda<sup>188</sup> required agar gel electrophoresis at pH 6.2. Abnormal electrophoretic behavior and even chromatography, however, cannot be relied upon to reveal all hemoglobin abnormalities (Chapter 24). In the last analysis, altered oxygen affinity of the blood, as measured by the partial pressure of oxygen at which hemoglobin is half saturated ( $P_{50}$ ), must be measured. For example, in Hb Olympia,<sup>217</sup> a battery of electrophoretic and chromatographic methods failed to demonstrate a hemoglobin abnormality. It was the reduced  $P_{50}$  that revealed the increased oxygen affinity of the blood. By appropriately excluding an alteration in the intracellular content of diphosphoglycerate (DPG), the one other type of abnormality that can cause altered oxygen affinity, an abnormal hemoglobin remained as the only explanation of the abnormal red cell function. Appropriate studies then revealed the nature of the amino acid substitution in Hb Olympia.

The normal effect of pH on oxygen affinity (Bohr effect) (Chapter 3) is reduced by a number of these mutations; this tends to shift the oxygen hemoglobin dissociation curve to the left. In addition, in the great majority of these mutations, impaired interaction between the four hemoglobin subunits ("heme-heme" interaction) was present. This effect is indicated by a change in the oxygen dissociation curve to a more hyperbolic and less sigmoid shape (Fig. 30-7). The physiologic effect of these changes is to impair the release of oxygen in the tissues. How this functional impairment is produced has been the subject of interesting studies of the effects of the amino acid substitutions on the quaternary structure of hemoglobin (Chapter 4). The location of all but three of the substi-

Table 30-3. Mutant Hemoglobins with Increased Oxygen Affinity and Degree of Associated Erythrocytosis

Name & Reference	Helix & Amino Acid Substitution	Abnormal Hemoglobin (%)	Electrophoretic Mobility	P <sub>50</sub> (mm Hg)	Bohr Effect	Heme-Heme Interaction (n <sup>1</sup> )	Hb Concentration (g/dl)	
							Male	Female
Olympia <sup>217</sup>	β 20(G2) val → met	40	Normal	6.8(8.5-9.1)H 18.6(26.8)B		2.6	21	
Heathrow <sup>221</sup>	β 103(G5) phe → leu	7	Normal	9.5-10.4(20-23)B	† 1% normal	1.2	21	16-17
San Diego <sup>111</sup>	β 109(G11) val → met	40	Normal	6.5-7.3(11-17)H 16-4(26.8)B		2.1		17-18
Involving the α <sub>1</sub> β <sub>2</sub> contact								
Hiroshita <sup>14, 212</sup>	β 37(G3) try → ser	40	Slow		Reduced	1.48	13-14	10-15
Chesapeake <sup>110</sup>	α 92(FG4) arg → leu	25-35	Fast	18-20(28)B	Normal	1.4	15-20	15-18
J Capetown <sup>115, 201</sup>	α 92(FG4) arg → gln	35	Fast	8.5(8.9)H	Normal	1.9	14	15-17
Malmö <sup>112, 116</sup>	β 97(FG4) his → gln	48	Fast	13-15(29-31)B	Normal(?)	1.5	17-21	14-18
Yakima <sup>202, 210</sup>	β 99(G1) asp → his	38	Slow	12(26)B	Normal	1.1	17-23	18-18
Kempsey <sup>215</sup>	β 99(G1) asp → asn	48-50	Slow	13.5(33)H	Present	1.1	19-21	17-20
Ypsilanti <sup>116</sup>	β 99(G1) asp → tyr	50	Slow	17.0(27)B			15-19	12-18
Brighton <sup>117</sup>	β 100(G2) pro → leu		Normal		Normal	SI dec	17-20	15-18
Involving the carboxy terminal								
Rainier <sup>105</sup>	β 145(HC2) tyr → cys	30	Slow†	11-13(30-31)H	Normal	1.2	15-21	18-21
Bethesda <sup>118</sup>	β 145(HC2) tyr → his	45	Slow†	11.5(28.8)B	Reduced	1.1	20	18
Hiroshima <sup>116</sup>	β 146(HC3) his → asp	50	Fast	4.6(9.2)H	Reduced	2.2		11-17
Little Rock <sup>117, 213</sup>	β 143(H21) his → gln		Fast†		Normal	2.9	23	

\*Values obtained in blood (B) or unfractionated hemolysates (H). Values from normal controls given in parentheses. Data from Stamatoyannopoulos <sup>214</sup>

†n is derived from the Hill equation. The normal value is about 3.0

‡Agar (Reimer) is also alkali resistant

arg = arginine; asn = asparagine, asp = aspartic acid, cys = cysteine, his = histidine, gln = glutamine, leu = leucine; met = methionine, phe = phenylalanine;

pro = proline, ser = serine, try = tryptophane; tyr = tyrosine

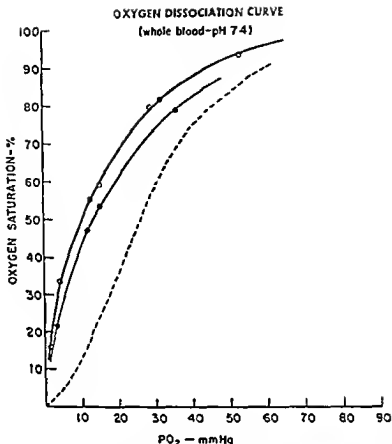


Fig 30-7 Whole blood oxygen dissociation curves for hemoglobins Yakima (O) and Rainier (●) as compared to the normal adult human hemoglobin (dashed line) (Modified from Adamson,<sup>181</sup> courtesy of the author and Henry M Stratton Inc)

tutions has been either in a position that could interfere with  $\alpha_1\beta_2$  contacts or at the carboxy terminal end of the  $\beta$ -chain (Fig. 30-8). It has been postulated that the equilibrium between oxyhemoglobin and deoxyhemoglobin may be altered to favor the oxy-form if: (1) the mutation introduces a bigger side chain that is a misfit in the deoxy-form; (2) a concentration of groups of equal charge results that repel each other in the deoxy-forms; (3) the amino acid substitution leads to the loss of a hydrogen bond stabilizing one or the other of the two quaternary structures; or (4) there is impairment of the binding of 2,3-DPG in deoxyhemoglobin. The last is the situation with Hb Little Rock.<sup>187,213</sup>

As illustrated in Figure 4-17 (page 177), it has been shown that normally, on oxygenation, the contact area at the  $\alpha_1\beta_2$  interface shifts from one dovetailed position to another. Eight of the abnormal hemoglobins associated with increased oxygen affinity involved this interface (Table 30-3). In Hb Yakima<sup>202,210</sup> and in Kempsey,<sup>215</sup> replacement of aspartate G1 $\beta$  may cause an intra-chain shift in the normal relations between the F and G helices and the heme group, or the substituted side chain may disturb the region of contact between nonpolar residues of the  $\alpha$ - and  $\beta$ -chains in a way to favor the oxy structure.

With reference to substitutions at the carboxy terminal (Fig. 30-8), Perutz has shown

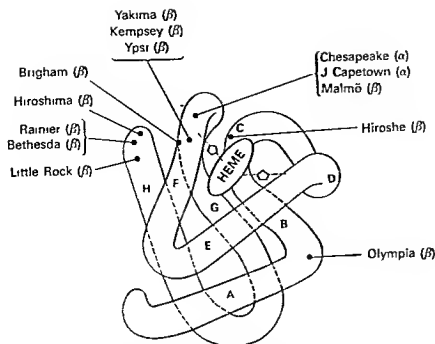


Fig 30-8 The approximate structure of the  $\alpha$ - and  $\beta$ -chains of hemoglobin showing the positions of known substitutions leading to increased oxygen affinity and secondary erythrocytosis

that the position of the penultimate tyrosine changes on oxygenation (Chapter 4). In Hb Bethesda,<sup>188</sup> the substitution of histidine for the penultimate tyrosine of the  $\beta$ -chains may prevent adequate stabilization of the deoxy tetramer; the partially ionized histidine is unable to occupy the hydrophobic pocket and, as a consequence, formation of the oxygen-linked salt bridges of the  $\beta$ -carboxy terminal histidine may be prevented. In Hb Rainier,<sup>189,205</sup> the penultimate  $\beta$ 145 cysteine residues may form intramolecular disulfide bonds with the  $\beta$ 93 cysteine residues and these may change the conformation around the tyrosine residues with resulting alteration in the  $\alpha\beta$  contact. Perutz suggests that replacement of histidines H 21(143) by aspartates would generate a pair of competing negative charges that would bind the imidazoles of histidines HC 3(146)  $\beta$  in both the oxy and deoxy forms, thus keeping their pKs permanently high and inhibiting their contribution to the Bohr effect.<sup>212</sup>

Whether or not erythrocytosis develops depends on a number of factors, eg, (1) the

proportion of abnormal hemoglobin; (2) interaction between normal and abnormal hemoglobins in the heterozygous state during binding with oxygen; (3) reactivity of the abnormal hemoglobin with organic phosphates present in erythrocytes, such as 2,3-DPG; (4) the magnitude of change in oxygen affinity of the hemoglobin; and (5) the stability of the hemoglobin. It is possible also that, in certain instances, the influence of other factors, such as blood loss, had not been ruled out as an explanation for lower hemoglobin levels than would otherwise have been expected.

Erythropoietin levels in patients with this type of hemoglobinopathy usually are not increased. This can be readily explained, however, if one assumes that a new homeostatic equilibrium has been achieved; at the time of study, the tissue anoxia has already stimulated erythropoietin production, and this in turn has induced erythrocytosis. When such patients were bled to normal hematocrit levels, reticulocytosis occurred and increased erythropoietin excretion developed;

as these individuals returned to their usual, compensated state of erythrocytosis, erythropoietin excretion dropped to normal.<sup>180</sup>

Cyanosis rather than erythrocytosis is characteristic of patients with hereditary methemoglobinemia<sup>236</sup> due to enzymatic defects that result in decreased reduction of methemoglobin (Chapter 31). Mild compensatory erythrocytosis may occur but this is inconstant. Likewise, in the Hb M disorders (page 1015), erythrocytosis is rare even in those due to substitution in the  $\beta$ -chain in which increased oxygen affinity has been observed. The absence of erythrocytosis may be explained by the fact that these variants are somewhat unstable.

### Acquired Abnormalities of Hemoglobin

A number of drugs and chemicals, such as nitrites, nitrates, aniline derivatives, sulfonamides, and various amines and nitrobenzene types of compounds, will produce toxic levels of methemoglobin and/or sulfhemoglobin in the blood of even normal persons.<sup>231,234,238</sup> Individuals with defects in methemoglobin reduction are especially sensitive to such agents<sup>232,236</sup> (Chapter 31). To our knowledge, clear erythrocytosis has not been demonstrated in patients with toxic methemoglobinemia. However, an increase in hemoglobin above pretreatment levels was seen in patients with sickle cell anemia given paraminopropiophenone in doses sufficient to maintain a methemoglobin level of between 20 to 30%.<sup>230</sup> Occasionally, in heavy smokers, carboxyhemoglobin concentration may reach sufficiently high levels (4 to 6.8%) to produce absolute erythrocytosis.<sup>239</sup> In phosphorus poisoning, especially in persons working in the match industry, polycythemia was described,<sup>240</sup> but this may have been merely relative erythrocytosis resulting from acute liver damage.

### Tumors and Miscellaneous Disorders<sup>270</sup>

Erythrocytosis has been described in association with a variety of neoplasms, cysts, and vascular abnormalities (Table 30-4).

**Table 30-4. Reports of Erythrocytosis Associated with Tumors and Cysts<sup>270</sup>**

	Number of Cases
<b>Adrenal and renal disorders</b>	
Hypernephroma	118
Cystic kidney disease	35
Hydronephrosis	14
Hepatocellular carcinoma	63
Cerebellar hemangioblastoma	50
Leiomyosarcoma of uterus	23
<b>Rarely (1 to 5 case reports)</b>	
Renal adenoma, hemangioma, sarcoma, ischemia, and renal artery stenosis	
Pheochromocytoma	
Hepatic hamartoma	
Wilms' tumor	
Myxoma of the atrium <sup>241</sup>	
Carcinoma (gastric, bronchogenic, prostatic, ovarian)	

### Hypernephroma and Renal Disorders<sup>270</sup>

Erythrocytosis has been reported in association with hypernephroma in 118 patients and it is estimated that 1 to 4% of patients with hypernephroma may develop erythrocytosis.<sup>270</sup> Erythrocytosis also has been reported in occasional patients with renal carcinoma, sarcoma, hemangioma, adenoma and Wilms' tumor,<sup>270</sup> renal cysts, hydronephrosis, or polycystic kidneys,<sup>233,254,258,270</sup> and, in one patient with renal ischemia due to disease of the extrarenal vasculature.<sup>261</sup> In a number of subjects a true increase in red cell mass was demonstrated. The rate of plasma iron disappearance was rapid and plasma iron turnover was increased, as in polycythemia vera.<sup>254</sup> When the lesions were removed the erythrocytosis disappeared. Extracts of the removed tumor or cyst were found to have erythropoietic activity in a number of instances, and when the tumors recurred erythrocytosis reappeared. However, in other patients with erythrocytosis no increase in erythropoietic activity could be demonstrated in the serum, cyst, or tumor. Furthermore, erythropoietic activity has been found in renal tumors or cysts removed from patients



without erythrocytosis.<sup>270</sup> Thus, in most but not all cases studied, erythropoietin or a precursor appeared to have been synthesized in the tumor or cyst or perhaps in compressed, adjacent normal tissue, and released into the circulation or concentrated in the lesion. Experiments in rabbits suggested that pressure on the renal parenchyma may cause erythrocytosis.<sup>263</sup>

### Hepatocellular Carcinoma<sup>270</sup>

Erythrocytosis also has been reported in a number of persons with hepatocellular carcinoma. An incidence of 10% was reported in one series of 176 consecutive cases.<sup>262</sup> In another series of 213 autopsied subjects whose death was due to hepatic carcinoma, in 9.4% the hemoglobin levels were above 16 g/dl, in 2.8% they were above 18 g.<sup>251</sup> Most of these liver carcinomas developed in patients with cirrhosis; consequently, splenomegaly was not unusual. Measurements of plasma volume demonstrated increased values, as is often the case in uncomplicated cirrhosis, but the red cell mass also was increased.<sup>262</sup> The rapid growth of this tumor leads to death within a few months and, as a result, few patients have been operated upon and no remission of the erythrocytosis has been reported.<sup>270</sup> An interesting case in which elevated plasma erythropoietic activity was demonstrated but no such activity could be found in the liver led to the finding of increased erythropoietin substrate in the tumor.<sup>255</sup>

### Cerebellar Vascular Tumors

About 50 cases of cerebellar vascular tumor with associated erythrocytosis have been reported.<sup>259,270</sup> Usually platelet and leukocyte concentrations were normal; the red cell mass was increased in some but not all of these patients.<sup>270</sup> The spleen was not enlarged. In several instances the presence of erythropoietic activity in tumor tissue or cyst fluid was demonstrated.<sup>250,259,270,272</sup> The removal of the tumor was associated with a return to normal hemoglobin levels in all 26 patients who were followed for a sufficient

period. Recurrence of tumor was accompanied by erythrocytosis in a few patients.<sup>270</sup>

### Leiomyoma of the Uterus<sup>270</sup>

Twenty-three cases of erythrocytosis in association with uterine myomas occurring both before and after menopause have been reported. In nearly all of these patients the tumor was large, reaching to the umbilicus or above.<sup>270</sup> In 18 of 20 patients who survived hysterectomy the erythrocytosis subsided. It is not clear whether the erythrocytosis was due to anomalous erythropoietin production,<sup>275</sup> interference with ventilation, or renal or ureteral compression caused by the large tumors, since few cases have been adequately studied.<sup>270</sup>

### Other Tumors<sup>24,270</sup>

In addition to the above, rare instances of erythrocytosis in association with a variety of other tumors have been reported (Table 30-4). From most of the case reports it is not possible to ascertain whether the erythrocytosis was relative or absolute, whether there was coincidental polycythemia vera, or whether the erythrocytosis had been caused by low arterial oxygen tension.<sup>270</sup>

It is well known that erythrocytosis with features suggesting polycythemia vera, namely, plethoric facies and leukocytosis, may be found in patients with Cushing's syndrome or primary aldosteronism.<sup>263</sup> These signs also have been observed in association with luteomas of the ovary.<sup>257</sup> Some degree of polycythemia can be produced by the administration of adrenal corticosteroids in large doses. Polycythemia has been observed in mice with masculinizing tumors (luteomas).<sup>257</sup>

### Pathogenesis of Erythrocytosis Associated with Tumors and Cysts

The erythrocytosis observed in association with tumors and other disorders discussed above has been termed *inappropriate*, in the sense that it occurs neither in response to

recognizable tissue hypoxia nor in association with a hemopoietic disorder. As indicated above, in many instances the erythrocytosis has been shown to be related to the production of erythropoietin. The erythropoietin appears to be identical with normal, physiologic, renal erythropoietin, for it is inactivated by erythropoietin antibody.<sup>273</sup> Depending on the circumstances under which the erythrocytosis has developed, a variety of mechanisms may have been responsible for the production of the erythropoietin. Knowledge in this field still is very meager. On the basis of what is known at this time, tissue anoxia induced as the result of pressure from an enlarging mass offers an acceptable explanation, but more intriguing is the implication that various tissues may be capable of producing erythropoietin or related substances under certain circumstances.

### Benign Erythrocytosis

The term "benign" or "primary erythrocytosis" was used to denote erythrocytosis not associated with leukocytosis, thrombocytosis, or splenomegaly, but characterized by increased red blood mass and total blood volume, and erythroid hyperplasia of the bone marrow, with no evidence of an underlying cause. The course was described as milder than that in polycythemia vera. It has been suggested that some patients with these findings may have a very mild form of polycythemia vera or the erythrocytosis may be due to an as yet unidentified cause; others may be at the extreme end of the normal distribution curve (page 977). The discovery of erythrocytosis associated with hemoglobins with high oxygen affinity may explain some of these cases (page 982). Other cases may have been instances of "inappropriate erythrocytosis," unrecognized as such because they were studied before it was appreciated that renal cysts and some tumors may produce erythrocytosis (page 986).

### "Benign Familial Erythrocytosis"

Scattered in medical literature are reports of families in which two or more members

had polycythemia. A review<sup>269</sup> indicated that, in addition to the dominantly transmitted hereditary forms caused by a hemoglobinopathy (page 982), there are other varieties of transmission, both dominant and recessive. The dominant forms include those in which there may be a defect in the regulation of DPG and still another variety of unknown etiologic background.<sup>269</sup>

The recessive forms have been observed in children or adolescents. In these the degree of erythrocytosis has been very striking, with hematocrit values greater than 0.71 l/l, whereas in the dominantly transmitted type, hematocrit values of 0.45 to 0.60 l/l have been found. Clinically, however, the erythrocytosis has been equally benign in both types.<sup>269</sup> Several instances of recessive familial erythrocytosis have been shown to be the consequence of a cellular defect in the regulation of production of erythropoietin or of its precursor substances.<sup>219,276</sup>

## Polycythemia Vera (Erythremia)

### Definition

Polycythemia vera is a disease of insidious onset, chronic course, and unknown cause. It is characterized by a striking, absolute increase in the number of red blood corpuscles and in the total blood volume, and usually by leukocytosis, thrombocytosis, and splenomegaly. The bone marrow is hyperplastic. The skin has a peculiar reddish-purple color and a variety of vasomotor and neurologic symptoms are manifest.

*Synonyms* include polycythemia rubra vera, splenomegalic polycythemia, Vaquez's disease, Osler's disease, polycythemia with chronic cyanosis, myelopathic polycythemia (Weber), erythrocytosis megalosplenica (Senator), and cryptogenic polycythemia (R. C. Cabot).

### History

In 1892, Vaquez<sup>240</sup> described persistent polycythemia, as distinguished from relative

and transient forms, in a man whom he took to have a congenital cardiac lesion even though there were no auscultatory signs. At autopsy, one year after the patient was first examined, his heart was found to be normal. The writings of Osler in 1903 and 1908<sup>327</sup> crystallized the clinical picture of the disease, and Türk,<sup>329</sup> in 1904, called attention to the occurrence of leukocytosis as well as to immature forms of cells of the red and white series, thus suggesting a hyperplastic disorder of blood formation. Excellent reviews of the literature have been published,<sup>21,62,311</sup> but little has been added to the clinical description since the earliest publications.

### Epidemiology

Polycythemia vera is much less frequent among Negroes than Caucasians<sup>320</sup> and is comparatively common in Jews.<sup>321</sup> Thus, in one reported series of 197 cases, in a hospital in which Negroes represented approximately 16 to 25% of all the patients,<sup>304</sup> only 7 of the patients with polycythemia vera were Negroes. Only 12 or so well-documented cases in Negroes have been reported.<sup>24,342</sup> The high incidence among Jews was first noted by Turk and is supported by the data in several series of cases.<sup>304,321,332</sup> The ratio in Jews, as compared to that in other persons, chiefly of European stock, is 2:1 or higher.<sup>320</sup> There is little to support the claim<sup>311</sup> that those affected tend to be of slender body build.<sup>304</sup>

Males are somewhat more commonly affected than females.<sup>320</sup> On the basis of a number of reports,<sup>301,320,339</sup> some of which diverge a good deal from one another, one would estimate the male to female ratio as somewhat less than 2:1, even 1.2:1.<sup>304,320</sup> The sex incidence and the age at time of diagnosis in 386 cases reported in three large series of subjects<sup>301,304,336</sup> are shown in Figure 30-9. The onset of symptoms in classic cases is in middle or later life.<sup>325</sup>

Occasional cases of idiopathic polycythemia have been reported in children.<sup>291,304,318</sup> and, of these, some were more suggestive of polycythemia vera<sup>291,318</sup> than others.<sup>304</sup> In many of these cases subsequent evaluations

indicated that most were probably cases of "benign familial erythrocytosis" (page 988).

### Pathogenesis

The symptoms and signs of polycythemia vera can be attributed in large part to the expanded blood volume and vascular space and to the slowing of the blood flow as a result of the increased viscosity of the blood.<sup>10,13</sup>

The cause of the disorder is unknown. There is no evidence that the oxygen-combining power of hemoglobin is altered nor is tissue respiration increased.<sup>311</sup> The suggestion that this disease is the compensatory result of anoxemia of the bone marrow was based on the presence of capillary thickening and subintimal and adventitial fibrosis of small vessels in the bone marrow.<sup>332</sup> However, direct measurements of bone marrow oxygen saturation gave normal values,<sup>525,527</sup> and the lack of elevated serum or urinary erythropoietin levels<sup>1,2</sup> also contradicts this hypothesis. In view of the increased production and turnover of erythrocytes, neutrophils, and platelets (page 994), and the hypercellular marrow, it appears more likely that there is abnormal cell production, perhaps beginning at the level of an uncommitted stem cell.

### Symptomatology

#### Onset

It is evident from the history in most cases that the disorder probably has been present for a long time. The presenting complaint may be headache, dizziness, ringing in the ears, or visual disturbances, or there may be dyspnea, lassitude, or weakness. Although the color of the skin is often acknowledged to have been unusual for a long time, this complaint alone rarely brings the patient to the physician. Skin and mucous membrane hemorrhages are not uncommon and these, or a sense of weight or swelling in the abdomen due to enlargement of the spleen, may be the initial symptoms. There may be such a multiplicity of symptoms that neurasthenia is

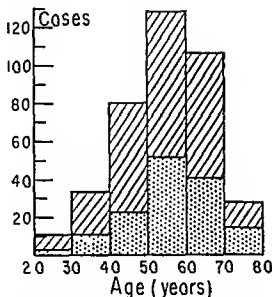


Fig 30-9 Age and sex of 386 patients with polycythemia vera.<sup>301,304,338</sup> Males are represented by hatched columns; females by dotted columns.

suspected. On the other hand there may be no complaints whatever, the polycythemia being discovered accidentally.

### Skin and Mucous Membranes

The color of the face is not like that of ordinary cyanosis but more nearly resembles that of a chronic alcoholic or that produced by blushing or exposure to a warm fire. This "rubor" may be so intense that it produces a startling appearance. The face, particularly the lips, cheeks, and tip of the nose, the ears, and the neck show this color (Fig. 30-10), but the skin of the trunk is not usually so strikingly affected. The distal portions of the extremities exhibit these changes more than the proximal portions and may be more truly cyanotic. The degree of red or blue depends upon the state of dilatation of the peripheral vascular network and upon the speed of circulation through these areas, since these factors determine the quantity of reduced hemoglobin present.<sup>318</sup> The delayed, sluggish peripheral circulation no doubt accounts for the sensitivity to cold, of which these patients may complain.

Ecchymoses of various sizes are common. In one patient, following the application of a mustard plaster, a hemorrhage that soon included almost the entire skin surface was observed<sup>345</sup>; and in another patient, ecchymosis almost as extensive followed a breast operation. Red or dark-violet spots or brownish pigmentation of the skin may be found and a great variety of skin lesions<sup>297,331</sup> have been observed (dry skin, eczema, acne-form or urticarial changes, acne rosacea, acne urticata,<sup>205</sup> urticaria pigmentosa,<sup>205</sup> and even a nodular eruption similar to the specific infiltration found in leukemia).<sup>310</sup> Purpura was observed in 8% of one series of 163 polycythemia vera patients.<sup>339</sup>

The eyes may appear bloodshot. The mucous membranes are a deep raspberry-red. Epistaxis and bleeding of the gums are common.

A common complaint is intense itching after a bath. This may be so troublesome that bathing with hot or even warm water is avoided. Less frequently, a similar reaction occurs following the use of cold water. This complaint tends to disappear as the polycythemia is treated, but returns with relapse.



Fig 30-10 Photograph of a drawing (original in color) of one of Osler's cases of polycythemia vera.

Reddening, swelling, and pain (erythromelalgia) may occur,<sup>300</sup> especially in the extremities. Club-like thickening of the terminal phalanges is uncommon.

### *Cardiovascular System*

Cardiac symptoms are not particularly prominent and cardiac hypertrophy is more frequently absent than present. The circulatory minute volume is reduced and the velocity of blood flow is greatly lowered,<sup>296</sup> but the cardiac output and work are normal.<sup>27,292</sup> The skin capillaries are distended and the capillary loops are enlarged. Vascular disease<sup>324</sup> is very common, and vascular accidents are frequent and in many instances are the cause of death. Venous thromboses occur in many of these patients and varicosities and phlebitis are often observed. Moderate or marked thickening of the peripheral arteries is found, and coronary thrombosis, claudication without occlusion, arterial occlusion with gangrene, acroparesthesia, the Raynaud syndrome, and thromboangiitis obliterans have been described.<sup>300</sup>

It has been stated that hypertension is so unusual in polycythemia vera that its presence indicates that the polycythemia is secondary in type.<sup>148</sup> This view seems extreme, for one might expect hypertension in a proportion of subjects whenever a chronic disease of middle and late life is involved. In one series of 20 patients,<sup>303</sup> systolic blood pressures greater than 140 mm Hg were found in 11 (55%) of the group. Our experience is similar (42%). In another series,<sup>315</sup> in one third of the 33% of patients in whom elevated blood pressures were present, the pressure returned to normal following successful treatment of the polycythemia.

### *Gastrointestinal System*

Besides feelings of fullness, thirst, gas pains, belching, and constipation, peptic ulcer, hemorrhage, or thrombosis may occur. Duodenal ulcer has been found in as many as 8% of patients with polycythemia vera, almost four times the number in a control

series.<sup>344</sup> In another series of 125 patients, duodenal ulcer was found in 16% and gastric ulcer in 7%.<sup>341</sup> It has been suggested that these ulcers follow thrombosis in the vessels of the first part of the duodenum and are produced by the action of the digestive juices upon the area of local necrosis.<sup>298</sup> Hemorrhage from varices in the esophagus, stomach, or bowel may be massive.<sup>293</sup>

Thrombosis in the mesenteric veins and arteries may be mistaken for peritonitis or the perforation of an ulcer.<sup>312</sup>

Enlargement of the liver is frequent (40% or more of subjects,<sup>335</sup> 50% in our series). Cirrhosis of the liver has been reported in a number of instances and some writers have distinguished, without much justification, a separate group of patients with liver cirrhosis (Mosse syndrome).<sup>323</sup> Occlusion of the hepatic veins (Budd-Chiari syndrome) has been observed.<sup>305,330</sup>

### *Splenomegaly*

Splenomegaly occurs in at least three fourths of these patients<sup>301,339</sup> (90% of our erythremia patients). The size of the spleen varies greatly in individual patients and occasionally it may even extend to the pelvic brim.<sup>303</sup> It is usually quite hard and smooth. There may be pain in the splenic region and, following infarction, a friction rub can be heard in this area. It has been assumed that polycythemia antedates the enlargement of the spleen and that engorgement of this organ with blood is the chief cause of the swelling.

### *Respiratory System*

Dyspnea on exertion of great degree is common and hoarseness is not unusual. Respiratory infections are easily acquired by these patients. Massive hemoptysis or hemothorax may occur. Roentgenograms usually reveal prominent vascular markings in the thorax.

In most patients with polycythemia vera the arterial oxygen saturation has been found to be normal, even when the hemoglobin levels have been high, indicating that the high

viscosity of the blood does not prevent the normal saturation of the blood with oxygen.<sup>370,372,413</sup> Breathing oxygen raises the oxygen saturation of the arterial blood to a degree comparable to that observed normally, and the oxygen dissociation curve is normal. High diffusing capacities which were reduced following phlebotomy were found in a number of patients with polycythemia vera.<sup>350</sup> Nevertheless, in another study, hypoxia as evidenced by a low arterial oxygen tension and saturation was observed in the absence of demonstrable coexistent cardiorespiratory disease.<sup>392</sup> In these individuals, ventilation/perfusion ratios were altered and the diffusing capacity was low. It was postulated that this might be the result of an alteration of the pulmonary vasculature due to unrecognized thromboembolism. From these observations it would appear that the measurement of oxygen saturation and tension as a means of differentiating primary and secondary polycythemias could be misleading.

### *Genitourinary System*

Vesical, vaginal, and uterine bleeding have been recorded, as well as a nontraumatic perirenal hematoma.<sup>317</sup> When there is hypertension, albuminuria and signs of renal disease may be found.

### *Neuromuscular System*

Headache is the most common neurologic symptom,<sup>333</sup> but lassitude, vertigo and giddiness, transitory syncope, insomnia, weakness, and a sensation of fullness in the head and numbness and tingling in the fingers, less often in the feet, are very common. As Osler remarked, these symptoms are like those of mountain sickness (page 977).

Visual disturbances are common and include transitory dimness of vision, or even temporary blindness, scotomas, specks and bright points in front of the field of vision, diplopia, and temporary paralysis of one of the eye muscles. On examination of the eye-grounds the vessels are observed to be engorged, tortuous, and irregular in diameter;

the veins are dark purple, the retina deeply colored. Papilledema has been observed and embolism of the central retinal artery has been reported.<sup>307</sup> The cerebrospinal fluid pressure may be increased.<sup>297</sup> Ringing and roaring in the ears are exceedingly common. Ménière's syndrome has been reported.

Vascular lesions of the brain constitute the most serious complication. Various resulting paralyses may be the first symptoms of the disease. Myoclonia, chorea,<sup>313</sup> grand mal attacks, and symptoms suggesting brain tumor, general paresis, and tabes have been associated with polycythemia vera, as well as narcolepsy, attacks of catalepsy, and psychic disturbances of various types (loss of memory, mental depression, confusion, hallucinations, slurring of speech).

Pains in the limbs may be very troublesome and severe. These have been attributed to the pressure on the bone by swollen, hyperplastic bone marrow. Curious paresthesias may be encountered and pruritus may be very distressing. Anatomic evidence of spinal cord changes has not, however, been found at autopsy.

### *Blood*

The venous blood is characteristically dark. It may be so thick that it is drawn up in a pipet with difficulty and spreads slowly between coverglasses. Red cell counts of  $7$  to  $10 \times 10^{12}/l$  are common when patients with this disease are first seen and values as high as  $12$  and even  $15 \times 10^{12}/l$  have been recorded.<sup>57</sup> It is difficult to accept some of the values reported. When red corpuscles are normal in size ( $87$  fl), there is "standing room" only for about  $11.5 \times 10^{12}$  cells/ $l$  and it is inconceivable that anyone could live with blood consisting of all cells and no plasma. We have seen the volume of packed red cells as high as  $0.86$  l/l blood in a patient with congenital heart disease and  $0.81$  l/l in one with polycythemia vera. The highest recorded is  $0.92$  l/l in a patient with polycythemia vera whose red cell count was  $10.37 \times 10^{12}/l$ .<sup>345</sup>

It is not unusual to find that, in patients

with marked polycythemia, MCV is reduced below normal. Usually this reflects the presence of iron deficiency. The average size in 22 of our patients was 80 fl. In one the MCV was as low as 61 fl. Somewhat more than  $16 \times 10^{12}$  cells/l as small as these could be packed in one liter.

Hemoglobin values as high as 40 g/dl ("240%") have been recorded, but the accuracy of such determinations must also be questioned. Values of 18 to 24 g/dl blood are more usual. The hemoglobin may not be increased in proportion to the increase in red cell count, this being the case when MCV and MCH are reduced. Sometimes the hemoglobin content of the red cells is reduced even more than their size (low MCHC), which indicates hypochromia. This is found particularly after large and repeated hemorrhages.

The individual red corpuscles usually appear quite normal. There may be slight anisocytosis, but poikilocytosis is unusual. Polychromatophilia and occasionally basophilic stippling may be found. An occasional normoblast may be observed in the blood smear and such a finding, in the presence of a relatively normal or definitely increased red cell count, should arouse suspicion of polycythemia vera. The reticulocyte count, in percent, is not significantly increased. Following a hemorrhage, however, the reticulocytes may be increased and a number of other immature forms of the red cell series may be encountered. If the hemorrhages are repeated, the morphologic appearance of the red corpuscles may be like that in iron deficiency anemia.

It is curious that neither Vaquez nor Osler appreciated the significance of the moderate or even marked leukocytosis, together with a "shift to the left" in the myeloid series of leukocytes that is often present in the disease named after them. Türk, in 1904, called attention to this significant finding, which suggests that the whole bone marrow is hyperactive rather than the erythropoietic tissue alone. The leukocyte counts were greater than  $10.0 \times 10^9/l$  in half of Osler's patients. In another series of 127 patients the white cell count was elevated in 84%.<sup>402</sup>

Leukocyte counts of  $25.0 \times 10^9/l$  are not uncommon<sup>62</sup> and values above  $50.0 \times 10^9/l$  have been recorded.<sup>371,407</sup> The myeloid leukocytes are relatively as well as absolutely increased, the metamyelocytes are increased in number, and 1 or 2% of myelocytes, sometimes more, are found. Myeloblasts usually are not observed. Basophil, eosinophil or monocyte concentrations may be increased. Leukocytes from polycythemia vera patients exhibit increased metabolic activity.<sup>372a</sup>

The blood platelets are frequently increased, usually in the 500 to  $1,000 \times 10^9/l$  range, but counts as high as 3,000 and even  $6,000 \times 10^9/l$  have been recorded.<sup>303</sup> Bleeding time and conventional coagulation parameters usually are normal, but the clot may retract poorly. It should be noted that the leukocyte and platelet counts are not always increased above normal in patients with otherwise typical disease. Such normal values were found in 20% of the patients in one series.<sup>336</sup>

It is not unusual, however, for morphologic and qualitative functional platelet abnormalities to be detectable. Platelets may appear to be abnormally large and even bizarre-shaped and megakaryocyte fragments sometimes are seen in the blood smear. The hemorrhagic complications of this disease suggest that a hemostatic defect may be present. Physical distention of the vascular bed is a plausible factor to explain excessive bleeding when it occurs. In addition, in some instances a fibrinolytic factor has been demonstrated.<sup>365</sup> Abnormal platelet thromboplastic function has been reported<sup>284,386</sup>, in a study of 29 patients, platelet factor 3 deficiency and excessive friability of the clot were found.<sup>360</sup> It was postulated that poor clot formation may result from the inadequate prothrombin-accelerating property of the platelets. Defects in platelet ADP release and aggregation<sup>373</sup> and in platelet adhesiveness<sup>409</sup> have been described.

Studies of fibrinogen turnover in three patients with erythremia indicated that fibrinogen was consumed in the course of chronic disseminated intravascular coagulation<sup>422</sup>; similar findings were observed in

several patients with erythrocytosis secondary to pulmonary insufficiency.

*Erythrokinetic studies* have shown active hemoglobin production, but otherwise findings have been diverse. Erythrocyte survival may be normal<sup>393</sup> or shortened<sup>401</sup> and splenic sequestration may or may not be present. As measured by the <sup>15</sup>N-glycine method, the rate of hemoglobin production was found to be about 2½ times the normal. There was a marked increase in plasma iron turnover rate.<sup>382</sup> This, incidentally, was not reduced by oxygen administration, as occurs when the polycythemia is due to anoxia.

A lengthened resistance span was described in studies of hypotonic saline fragility.<sup>383,398</sup> Increased serum bilirubin<sup>345,398</sup> and increased urine and stool urobilinogen<sup>401</sup> have been demonstrated in some patients with polycythemia vera, but, when allowance is made for the increase in the total amount of hemoglobin that must be degraded, fecal urobilinogen values rarely are increased above expected values and may, in fact, be somewhat reduced.

*Neutrophil kinetic studies* in patients with polycythemia vera whose neutrophil counts ranged from normal to  $23.9 \times 10^9/l$  showed a blood neutrophil pool that ranged from normal to 12 times normal, with increased margination and a normal or slightly prolonged half disappearance time. The blood neutrophil turnover rate (effective neutrophil production) was usually increased and varied from normal to 5 times normal mean values.<sup>362</sup>

*Platelet kinetic studies* in five patients with polycythemia vera in whom the platelet concentration was increased revealed effective production rates that ranged from 2 to 13 times normal. The marrow megakaryocyte mass was increased in all five patients.<sup>378</sup>

The *viscosity* of the blood may be 5 to 8 times greater than normal.<sup>62,315</sup> The *specific gravity* is 1.075 to 1.080, as compared with the normal range of 1.055 to 1.065. The degree of abnormality varies with the relative quantity of red corpuscles. The viscosity and specific gravity of the serum were found to be actually less than is normal.<sup>400</sup> The eryth-

rocyte sedimentation rate of polycythemic blood is greatly retarded.

**TOTAL BLOOD VOLUME.** This is characteristically increased. The enormous increase in blood, which distends even the smaller vessels of the whole body, accounts, no doubt, for many of the symptoms of this disease. In a group of 30 patients whose volume of packed red cells was 0.55 l/l or greater, the total red cell volume, measured by the <sup>32</sup>P-labeled red cell method, was 38.8 to 93.9 ml/kg body weight as compared with the normal average of 29.9 ml/kg.<sup>364</sup> In two thirds of these patients the plasma volumes were below the lower limits of normal and in none was the plasma volume above normal. Similar observations have been made by the <sup>51</sup>Cr method.<sup>336</sup> Because of variations in plasma volume, the volume of packed red cells gives only a rough indication of the size of the red cell mass.

### *Bone Marrow*

The marrow is dark red and very cellular. The hyperplasia involves all the marrow elements, however, so that the ratio of the different types of cells to one another is not strikingly different from the normal. The percentage of nucleated red cells may be moderately elevated.<sup>61,414</sup> These cells may be either orthochromic normoblasts or of less mature type, but megaloblasts are not found. There may be more myelocytes and myeloblasts than is normal and an unusual number of eosinophilic and basophilic leukocytes may be found. Megakaryocytes are sometimes more numerous than is normal and may be larger than normal.<sup>378</sup> In a series of 19 untreated patients, no marrow iron could be demonstrated.<sup>306</sup>

On biopsy, general hyperplasia with replacement of adipose tissue by hematopoietic cells is evident<sup>62</sup> and clumps of stem cells and basophilic erythroblasts are seen.<sup>368</sup>

### *Other Findings*

The *urine* may be normal, but albuminuria is found not infrequently and, less often, casts



are present.<sup>62</sup> The increased urobilinogenuria found in some of the patients has been mentioned previously. Studies of renal hemodynamics suggested that glomerular filtration, in spite of the decreased fraction of plasma in the blood, is kept at almost normal values by an increase in renal blood flow and in the proportion of plasma filtered.<sup>412</sup>

The amount of uric acid in the serum may be normal or increased. Hyperuricemia was present in 70% of one series of 127 patients. In another series of 47 patients, values from 2.8 to 11.7 mg% were found (average 6.6 mg%).<sup>62</sup> Secondary gout occurs in 5%<sup>304,336</sup> or more of these patients.<sup>336,341,402,496</sup> The occurrence of hyperuricemia in polycythemia vera is attributable to overproduction of uric acid.<sup>408,415</sup> When urinary uric acid was labeled cumulatively with <sup>15</sup>N-glycine, a sharp contrast was noted between the rapid peak of isotope incorporation into uric acid in primary gout and the slow incorporation in secondary gout.

A vitamin B<sub>12</sub> binding protein, which may be an altered form<sup>369</sup> of transcobalamin I (Chapter 4), has been found in the plasma of patients with polycythemia vera<sup>376</sup> and in a variety of conditions involving leukocytosis.<sup>369</sup> This may explain the observation that, whereas serum B<sub>12</sub> content may be within the normal range, or only moderately elevated, the capacity of the serum to bind additional vitamin B<sub>12</sub> added in vitro (unsaturated B<sub>12</sub> binding capacity, U B<sub>12</sub> BC) is increased.

Spurious hyperkalemia has been noted when the platelets have been greatly increased in number.<sup>399</sup> Hyperhistaminemia and hyperhistaminuria were reported in two thirds of a series of patients with polycythemia vera<sup>478</sup> and this may partly explain the pruritus often present (page 990). The basal metabolic rate may be increased moderately.<sup>367</sup> Gastric acidity may be normal or increased, or there may be anacidity.

### Cytogenetics

Few satisfactory reports of cytogenetic findings in untreated patients with polycythemia vera are available. Such as they are,

they are inconsistent. Some investigators have reported normal results.<sup>388,389</sup> Others found some degree of aneuploidy in 7 of 11 patients.<sup>387</sup> In still another study, an extra C-group chromosome was found in marrow cells of about 10% of the patients.<sup>396</sup> In treated patients, extensive chromosome abnormalities have been described.<sup>387,394</sup>

### Diagnosis

The characteristic color of the skin of patients with polycythemia vera may not be so striking that its significance is recognized at once, and the spleen is not always enlarged. Furthermore, the symptoms are so varied that a great number of diseases, particularly those of the cardiovascular and nervous systems, may be simulated. The first manifestations may suggest peripheral vascular disease (thromboangiitis obliterans, erythromelalgia), or gastrointestinal disease (peptic ulcer), gout, or some wholly unrelated disorder; or the symptoms may be so numerous and unrelated that neuroasthenia may be considered.

However, when the classic triad—ruddy cyanosis, splenomegaly, and polycythemia—is present, making the correct diagnosis should not be difficult. Furthermore, morphologic signs of accelerated erythropoiesis, such as occasional normoblasts in the blood smear and some polychromatophilia, often are present. In addition, as mentioned previously, leukocytosis, with moderate shift to the left, and thrombocytosis are found in 67 to 80% of the patients.

When splenomegaly and signs of bone marrow activity involving all three morphologic elements of the blood are present and the degree of polycythemia is not great, the possibility that one is dealing with an early stage of chronic myelocytic leukemia or of myelofibrosis must be considered. Rare cases of chronic myelocytic leukemia which, in their early stages, were characterized by slight polycythemia rather than anemia have been described.<sup>361,406</sup> Then again, if gastrointestinal bleeding or hemorrhage from another source has taken place and its severity has not been fully appreciated, the finding of

anemia together with leukocytosis, thrombocytosis, and splenomegaly may lead to a diagnosis of leukemia which only is recognized as mistaken after sufficient time has elapsed for red cell regeneration to restore the previous polycythemic levels. Further difficulties in differential diagnosis arise from the fact that, in polycythemia vera, the leukemoid manifestations may be so pronounced that the picture of chronic myelocytic leukemia is very closely simulated. This was true in 10% of one series of 163 cases<sup>138</sup> and in 40% of 127 cases in another.<sup>402</sup>

The *leukocyte alkaline phosphatase* (LAP) test (page 1279) may be of help in differentiating polycythemia vera with an associated leukemoid reaction from chronic myelocytic leukemia since values above normal may be found in the former and lower values are found in CML. Thus the LAP score was elevated on at least one occasion in all 74 patients with polycythemia vera tested in one series. Also the LAP score was elevated in two patients presenting with features suggesting chronic myelocytic leukemia in whom the subsequent picture was that of polycythemia vera.<sup>402</sup> In another series of 25 patients with polycythemia vera, high, abnormal scores were found in 20 and normal values were obtained in five, four of whom were in the early stages of the disease.<sup>406</sup> In a smaller series of 20 patients, followed with serial observations over a six-month period, during which they were in remission as a result of treatment, only eight subjects had elevated LAP scores; 11 fell within the normal range.<sup>361</sup> It has been reported that patients treated with busulfan had LAP scores that were lower than the average for the erythremic group.<sup>402</sup> In one patient with a low LAP score, the Ph<sub>1</sub> chromosome was demonstrated in the marrow<sup>361</sup>; after a 3½-year course closely simulating polycythemia vera this patient developed a rapidly evolving leukemic picture and died within a month.

Much greater difficulty in differential diagnosis arises when splenomegaly is not present and leukocytosis and thrombocytosis are not found; this, as mentioned earlier, may be the situation in perhaps 20 to 30% of

patients with polycythemia vera. In such patients the red cell mass should be measured, and if elevated the various causes of erythrocytosis, discussed in the first part of this chapter, must be considered. Pulmonary and cardiac function studies, a LAP test, measurement of  $P_{50}$  (page 982), and an intravenous pyelogram are then indicated and should help to establish the diagnosis. In most instances of erythrocytosis, normoblasts are not found in the blood and leukocytosis and thrombocytosis are not present. Thus, in a series of patients with congenital heart disease, slight leukopenia with a slight absolute decrease in the number of eosinophils, monocytes, and lymphocytes was found.<sup>141</sup> However, normoblasts have been described in the peripheral blood of patients with congestive heart failure<sup>432</sup> and, therefore, this finding alone cannot be taken as evidence of polycythemia vera. Even the coexistence of gout does not indicate that one is dealing with polycythemia vera since gout has been observed in erythrocytosis secondary to congenital heart disease.<sup>431</sup>

In view of the age incidence of polycythemia vera it may be expected that a certain proportion of the patients may also have chronic pulmonary or cardiac disease and these may be contributing in a minor way to the polycythemia. Measurement of red cell mass and total blood volume will help to separate absolute from relative polycythemia but this will not exclude the various causes of true erythrocytosis mentioned above. In these "secondary" forms of polycythemia, the total blood volume and red cell mass may be increased as much as in polycythemia vera.<sup>62</sup> The measurement of arterial oxygen saturation and tension (page 979) has not been as helpful in differential diagnosis as one would expect, but  $P_{50}$  measurement should distinguish cases due to increased oxygen affinity. The fact that various tumors may produce erythrocytosis adds to the diagnostic problems arising in some instances, since some tumors may not be demonstrated readily. An intravenous pyelogram should be performed to rule out the possibility of renal causes of erythrocytosis (page 986), but other causes

are less easily detected. The leukocyte alkaline phosphatase may be helpful, as already mentioned.

"Gaisböck's syndrome"<sup>54</sup> and "stress erythrocytosis" have been the source of considerable confusion. They were fully discussed in an earlier section of this chapter (page 976).

## Treatment

### General Remarks

As long as the cause of polycythemia vera is unknown, treatment must be symptomatic. A number of methods have been recommended to relieve the symptoms of this disorder. Relief of most of the symptoms is accomplished by lowering the red cell mass and total blood volume. This can be done by removing blood (venesection), by destroying red cells *in vivo* (phenylhydrazine), or by suppressing blood production in a variety of ways (irradiation; chemotherapeutic agents). In choosing a method of treatment the object should be to produce a reduction in the blood volume by means that: (1) have the smallest chance of causing harm; (2) permit the longest survival; (3) permit the greatest proportion of time at work and recreation; and (4) are least expensive and inconvenient for the patient. The choice of a method is relatively simple if the above objectives are kept clearly in mind.

### Venesection

Venesection offers prompt and effective restoration of the red cell mass and blood volume to normal. Many patients can be maintained in an essentially normal state by phlebotomy together with a few simple adjuvants, when necessary, to control hyperuricemia or pruritus.<sup>509</sup>

In criticism of venesection, it has been stated that the method is somewhat troublesome, leukocytosis and thrombocytosis are not controlled, and erythropoiesis is stimu-

lated by the blood loss. The last objection has not been substantiated<sup>445</sup>; presumably reticulocytosis does not occur after adequate phlebotomy because the blood is removed from the body and iron deficiency is induced.

Venesection has been used primarily for its immediate effects in relieving symptoms, especially vertigo, fullness in the head, headache, tinnitus, mental torpor, weakness, and pain in the bones, muscles, or joints. If 500 ml are removed repeatedly at one- to three-day intervals it is possible to reduce the blood volume to normal (usually after 6 to 8 units have been removed), thereby producing a remission that may last several, even 15, months.<sup>445</sup> In patients over 65 or in those with evidence of vascular complications,<sup>509</sup> smaller phlebotomies with infusion of plasma<sup>475</sup> or dextran<sup>16</sup> to maintain the blood volume while reducing the blood viscosity and red cell mass have been advocated. However, except in the presence of congestive failure or in the face of impending vascular thrombosis in patients with very high hematocrit values, as suggested by transient ischemic attacks or angina (page 976), too aggressive therapy is best avoided in this relatively benign disease.<sup>509</sup> Generally, patients are comfortable if the volume of packed red cells is brought to the upper levels of normal rather than to still lower levels. Venesection can be used as the sole therapeutic measure in perhaps two thirds of the patients,<sup>509</sup> at least for time. Good control can be maintained by one or two 500-ml phlebotomies every three or four months. In India, patients with polycythemia vera were successfully treated by producing hookworm infection and iron deficiency.<sup>468</sup> When phlebotomy is required more often than once every two months it is generally preferable to resort to other forms of therapy. Of those that will be described below, the agent most generally preferred is radioactive phosphorus.<sup>509</sup>

The employment of an iron-free diet<sup>498,509</sup> or other dietary regimens has not been very successful and imposes unnecessary hardship on patients with so chronic a disease as polycythemia vera.

**Phenylhydrazine**<sup>476,489,491</sup>

Phenylhydrazine is a base related to antipyrine and was prepared on a large scale from the anilin used commercially to produce antipyrine. It was introduced for the treatment of polycythemia vera by Eppinger and Kloss in 1918. The liquid form is unstable, but the crystalline hydrochloride is less easily decomposed unless exposed to air. The drug was given in capsules, usually in amounts of 0.1 to 0.3 g/day up to a total dose of 1.5 to 3.5 g.<sup>477</sup> It is no longer used in this way because of its gastrointestinal and other toxic effects and the danger of producing severe hemolytic anemia.<sup>485</sup> It rarely is used even as adjuvant therapy to maintain remissions. In doses of 0.1 g/day several times per week, however, the toxic effects are minimal. Phenylhydrazine also may be useful in patients in whom thrombocytopenia develops after radiophosphate therapy. In contrast to phlebotomy the products of destruction of the red cells (chiefly iron) remain within the body and are utilized again.<sup>438</sup>

**Irradiation**

Irradiation, in one form or another, has been used as a method of treatment for many years. Radiation was first directed to the spleen, without benefit.<sup>494</sup> Irradiation of the bones was first successfully applied in 1916.<sup>490</sup> Deep roentgen therapy, irradiation of the whole body ("spray therapy," "total radiation"),<sup>501</sup> and radium as well as thorium-x have been used.<sup>497</sup> Irradiation is relatively slow in action, requires special apparatus as well as skill, and the effect produced, if greater than expected, may be irreparable. On the other hand, the remission produced with successful irradiation may be relatively prolonged. Of the forms of irradiation therapy, most satisfactory is radioactive phosphorus, which causes no nausea or vomiting and can be administered easily.

**Radioactive phosphorus (<sup>32</sup>P)**<sup>495</sup> is usually provided as the dibasic sodium salt. It is soluble in water and, while it is effective

when given by mouth,<sup>513</sup> it has been used more successfully when given by vein.

<sup>32</sup>P passes to tissues that have a high phosphorus content and metabolize phosphorus rapidly. Its uptake by rapidly dividing cells is greater than that by normal cells. Since the physical half-life of this isotope is 14.3 days, steady irradiation of tissue takes place for several weeks.<sup>473</sup> Its concentration in bone makes <sup>32</sup>P particularly valuable in the management of hematopoietic disorders. Among these its value has been greatest in the management of polycythemia vera.

<sup>32</sup>P induces satisfactory clinical and hematologic remissions in polycythemia vera and *these have lasted as long as one to two or more years.*<sup>315,483</sup> As with roentgen therapy, the fall in the red cell count does not usually begin before 30 to 60 days after <sup>32</sup>P has been given. Care must be taken to avoid producing anemia, leukopenia, or thrombocytopenia. Unlike the effects of roentgen therapy, radiation sickness does not develop.

A recommended method of treatment is as follows: The VPRC is brought down to 0.55 l/l, or lower, by phlebotomies. The patient is then given between 3 and 5 millicuries (mCi) of <sup>32</sup>P, intravenously or 2.3 mCi/m<sup>2</sup>.<sup>448</sup> No additional <sup>32</sup>P is given for three months, in order to avoid cumulative effects. If the VPRC rises above 0.55 l/l in the interval, venesection can be performed. If, after three months, the VPRC still is above 0.55 l/l, a second injection of 1 to 4 millicuries is given. Examinations are repeated at three-month intervals. Some patients do not require a second injection or further phlebotomies for 6 to 18 months or longer. Perhaps 10% of patients will need a third injection. After this, it has been recommended that no further injections be given for at least a year, preferably 18 months.<sup>495</sup>

An analysis of 300 courses given to 139 patients showed that an average of 6.7 mCi had been given in a "course," as represented by a six-month period.<sup>62</sup> The majority of these patients were retreated within intervals of 6 to 10 months. In another series of 241 patients<sup>501</sup> the average dose required to pro-

duce remissions was 5.7 mCi in patients without leukocytosis or myeloid immaturity and as much as 8.3 mCi in others. The range of dose required was 3 to 21 mCi.

Some of the pros and cons of radiation as compared with other forms of therapy have been discussed previously (page 997). In addition, as large series of patients treated with radiophosphate have accumulated,<sup>436,440,506,509</sup> an incidence of leukemia of perhaps 10 to 15% has become apparent. It is not yet clear whether this is because patients so treated have survived long enough to develop leukemia, which some observers believe may be part of the natural evolution of this disorder,<sup>436,509</sup> or because it is a direct effect of irradiation treatment.<sup>323,440</sup> It is noteworthy that damage to chromosomes was shown to occur following treatment with <sup>32</sup>P, but this is not a consistent finding and aneuploidy has been described even in untreated subjects.<sup>337,394</sup>

When the incidence of leukemia in polycythemia vera patients treated with <sup>32</sup>P was first publicized, there was at first a trend against the use of this therapy. In time, however, after it was realized that this treatment is so simple and effective, morbidity is so low, and survival is so prolonged when the therapy is used wisely, <sup>32</sup>P came again to be regarded as a very useful therapeutic agent. A combination of phlebotomy and, when necessary, modest doses of radiophosphate (or perhaps other myelosuppressants)<sup>448</sup> provides a simple and effective mode of therapy in most patients. The median survivals in patients so treated have ranged from 10 to 14.5 years.<sup>62,483,506,509,511</sup>

Whether or not to take the risk of acute leukemia developing, perhaps as an ultimate (and as yet unproved) ill effect of <sup>32</sup>P therapy, must be a decision for the physician and his patient to make. Until data from a well-controlled clinical trial have become available or some other and better form of therapy has been devised, we believe that the use of <sup>32</sup>P in the treatment of polycythemia vera is justified in most cases. However, it has always been our practice to use the minimum

amount of <sup>32</sup>P that is practical in a given patient. This approach is now supported by the evidence that the risk of acute leukemia appears to be dose dependent.<sup>440</sup>

### Chemotherapy

In the past, various chemotherapeutic agents have been used for the treatment of polycythemia vera. Benzene was once used<sup>456</sup> but was found to be too toxic. Fowler's solution (potassium arsenite) was found to lower the blood count in some of the patients<sup>472</sup> as did nitrogen mustard,<sup>502</sup> triethylene melamine (TEM),<sup>499</sup> and thiotepea.<sup>471</sup> Given orally, TEM produced satisfactory remissions in 20 of 30 subjects, with remissions lasting eight or nine months. However, thrombocytopenia was a frequent complication. The antimalarial drug pyrimethamine was shown to have an effect in this disease.<sup>474</sup> A neutral piperazine (Vercyte) was also found to be effective.<sup>492</sup> However, none of these agents has survived the test of time.

Because of the possibility that <sup>32</sup>P may be responsible for the ultimate development of leukemia in patients so treated, as discussed under the foregoing heading, there has been a resurgence of interest in the management of polycythemia vera with chemotherapeutic agents.<sup>469,487,507,509</sup> Busulfan (4 to 6 mg/day given orally) has been the most preferred agent,<sup>467,509</sup> but has proved to be much more effective in controlling the leukocytosis and thrombocytosis than in the management of the erythrocytosis.<sup>467</sup> It tends to produce excessive and prolonged leukopenia and thrombocytopenia in many patients, and occasionally marrow aplasia and other complications such as skin pigmentation and pulmonary fibrosis.<sup>487</sup> Chlorambucil (6 to 8 mg daily) has been recommended for patients with normal or low platelet and leukocyte counts; cyclophosphamide (100 to 150 mg/day) also may be useful in these patients.<sup>487,509</sup> Melphalan produced excellent initial results in 24 of 27 patients; its side reactions were infrequent.<sup>487</sup> Other chemotherapeutic agents have also proved effective

in limited trials.<sup>469,507</sup> All such agents carry the disadvantage that their use requires much more frequent supervision than is necessary when <sup>32</sup>P is employed. In addition, it is known that these agents, like <sup>32</sup>P, may favor the development of somatic mutations. Occasionally, leukemia has been reported as developing in patients treated with busulfan.<sup>436,448</sup> It is still too early to ascertain whether use of this drug or of other chemotherapeutic agents will decrease the incidence of leukemia or the myeloid metaplasia-myelofibrosis transformations that develop after <sup>32</sup>P therapy. A prospective study under the sponsorship of the National Institutes of Health is under way and it is hoped that this will provide the answer to such questions.<sup>448</sup>

### *Splenectomy*

Splenectomy has been performed in a number of polycythemia vera patients. It is not only valueless, but may be harmful; death has followed in a number of instances.<sup>484</sup>

### *Treatment for Relief of Miscellaneous Symptoms*

Hematologic control, especially if accomplished by myelosuppressive therapy, usually relieves the various manifestations of polycythemia vera and improves the performance status of the patient. However, certain symptoms may persist to some extent and require treatment.

**PRURITUS.** The pruritus, upper gastrointestinal distress, and urticarial manifestations of polycythemia vera were found to correlate with increased levels of whole blood histamine, which, in turn, was roughly related to the basophil leukocyte count.<sup>478</sup> These clinical manifestations were controlled by the administration of a potent antihistaminic, cyproheptadine (4 mg three or four times per day), in 12 of 18 patients so treated.<sup>478</sup> This observation, if confirmed, would favor the use of myelosuppressive agents in the treatment of polycythemia vera since these produce a reduction in histamine levels whereas phlebotomy does not.

**HYPERURICEMIA.** The common occurrence of hyperuricemia and gout in patients with polycythemia vera has been mentioned earlier in this chapter. Because of the excessive urinary load of uric acid excreted by patients with "myeloproliferative disorders,"<sup>511</sup> urate may be precipitated in the kidneys, leading to stone formation or nephropathy. In one series, urolithiasis occurred in 40% of 44 patients with secondary gout as compared to 20% of 937 patients with primary gout.<sup>482</sup> An effective means of reducing uric acid production in polycythemia vera, other than by myelosuppression, is by the use of allopurinol in doses of about 300 mg per day.<sup>514</sup> However, although this drug has been given for longer than one year to some patients with minimal side effects,<sup>514</sup> its use should probably be restricted to short periods when the avoidance of uric acid deposition in patients starting on therapy is a major concern. For long-term management, adherence to the general principles of the treatment of gout is recommended.<sup>465,490</sup>

### *Course and Complications*

The course of polycythemia vera is chronic, but various complications may develop. When the high incidence of vascular complications is avoided by appropriate therapy the course usually is characterized by periods of remission with few, if any, symptoms, interspersed with more or less asymptomatic relapses that again respond to treatment. Intercurrent infections may be frequent, especially those of the respiratory tract. Bronchitis and emphysema may develop. Elevated levels of IgM and IgG may be found.<sup>439</sup> The association with duodenal ulcer, gout, hypertension, and cirrhosis of the liver has been mentioned previously. In patients with hypertension, chronic renal disease and arteriosclerosis are common. Albuminuria may be present, possibly as the result of blood stasis in the kidneys. In view of the frequent finding of an increased basal metabolic rate, it is of interest that hyperthyroidism has been reported in only one well-studied case.<sup>315</sup> Various other conditions associated with polycythemia vera have been

reported from time to time; these include paroxysmal hemoglobinuria,<sup>442</sup> agranulocytosis,<sup>444</sup> chronic lymphocytic leukemia,<sup>425</sup> lymphoma, multiple myeloma,<sup>435</sup> hemoblastic sarcoma,<sup>443</sup> and osteosclerosis,<sup>407</sup> as well as termination with a picture resembling that of aplastic anemia,<sup>428</sup> pernicious anemia,<sup>429</sup> or leukoerythroblastic anemia.<sup>446</sup> Termination of polycythemia vera in leukemia and in myelofibrosis will be discussed shortly. The "acute erythremia" of di Guglielmo is quite different from the disorder under discussion here. Its course is acute and the condition is characterized by anemia and marked erythroblastosis (page 1475).

Eventually, in perhaps 25% of polycythemia vera patients, there is a progressive reduction in erythrocyte survival, unaccompanied by adequately increased erythropoiesis, and myelofibrosis (Chapter 57) develops.<sup>430,436</sup> Extramedullary hematopoiesis takes place in the spleen and elsewhere, including the liver. A rising leukocyte count, often with increased immature myeloid forms, accompanies these changes and tear-drop forms and nucleated red cells appear in the blood.<sup>436</sup> The spleen may enlarge dramatically. About one third of such patients ultimately develop a picture simulating that of acute myeloblastic leukemia.<sup>436</sup> Others gradually become more anemic and thrombocytopenic and die of a variety of intercurrent complications in the "spent phase" of myeloid metaplasia.<sup>448</sup> Still other patients (perhaps 15%) develop an acute leukemia-like picture without preceding evidence of myeloid metaplasia or myelofibrosis.<sup>436</sup> Whether these changes are the result of irradiation therapy or reflect the natural evolution of the disease when early death from thrombosis or hemorrhage is avoided is moot, as was discussed earlier in this chapter. In certain studies the finding of abnormal chromosomes in about 10% of untreated patients has been cited to support the suggestion that a subgroup of patients with polycythemia vera possesses a propensity for cytogenetic accidents and perhaps these are the patients destined to develop leukemia.<sup>436,448</sup>

## Prognosis

The prognosis in polycythemia vera in the absence of treatment is not easily determined. In one review of 250 patients who died between 1933 and 1961<sup>426</sup> it was found that 50% of 49 untreated patients died within 18 months after the onset of the first symptom or sign (Fig. 30-11). Thrombosis was the cause of death in 100 of the 250 patients in that series, while death resulted from hemorrhage in only 15 patients. Myelofibrosis developed in 14, leukemia in 9, and other forms of anemia in 6. Half of the 62 patients treated with venesection died after 3½ years. Of the 48 treated by x rays, half were alive 12½ years after the first sign of the disease. This is consistent with median survival figures of 11.5,<sup>440</sup> 13.6,<sup>402</sup> and even 16<sup>506</sup> years reported by others. In another study it was found that thromboses occurred much less frequently (4.2%) in patients treated successfully with radioactive phosphorus<sup>415</sup> than prior to the introduction of this form of therapy when thrombosis occurred in approximately 25% of the patients.

The benefits of adequately controlling the disease can also be seen in the reduction of deaths and complications following surgical procedures necessary for associated problems. Thus, in 62 major operations performed in patients with polycythemia vera, the incidence of postoperative complications (mainly thrombosis and hemorrhage) in inadequately controlled patients was 83% as compared to 21% in patients with normal hemoglobin values. A long period of effective control preoperatively was associated with only 6% complications as compared to 33% for patients controlled for only a short time.<sup>449</sup> The incidence of postoperative deaths was also dramatically reduced from 37% to 5% as the result of adequate preoperative control.

Survival following treatment with <sup>32</sup>P has been the subject of much discussion and, unfortunately, there is no agreement. Osgood concluded that the survival time of <sup>32</sup>P-treated patients was about four years longer than for patients treated by other methods.<sup>325</sup> Others found no difference between the sur-

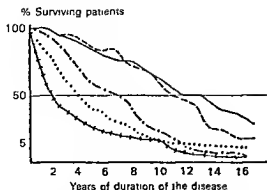


Fig 30-11 Percentage survival for patients with polycythemia vera

— untreated	49 patients
..... venesection only	62 patients
- - - - - $^{32}\text{P}$ and/or chlornaphazine	52 patients
- - - - - x-ray therapy only	48 patients
- · - · - various combinations of x-ray therapy	36 patients

(From Chievitz and Thiede <sup>426</sup> courtesy of the authors and the Acta Medica Scandinavica)

vival of patients treated with x ray or  $^{32}\text{P}$  as compared with those treated with other effective modes of therapy.<sup>402,433</sup> Modan and Lilienfeld,<sup>440</sup> on the basis of their survey, came to the same conclusion. The results of a well-controlled clinical trial on the basis of which a wholly reliable conclusion can be based have yet to be published.<sup>609</sup>

Although in one of the above-mentioned investigations no cases of leukemia were observed among 107 patients treated by radiation or in 117 treated without radiotherapy,<sup>433</sup> in Modan and Lilienfeld's and in Osgood's studies the incidence of acute leukemia as a terminal event was found to be higher among patients treated with irradiation than among those not so treated (perhaps 9:1).<sup>440</sup>

Whatever the solution to the questions raised above may be, there is no doubt that the life expectancy and well being of patients with polycythemia vera whose disease has been wisely managed have been greatly prolonged, as compared with the outcome of untreated patients. This is consistent with our experience.

## Pathology

The extreme plethora, the engorgement of all the organs with blood which flows, when the heart is removed, "as if from an inexhaustible spring,"<sup>526</sup> and the enlarged and thrombosed veins as well as the unusual color of the skin and the large and small hemorrhages in the skin, mucous membranes, brain, meninges, serous cavities, and the various organs make up a striking and characteristic picture.

The spleen is enlarged, smooth, moderately hard, and dark bluish-red and may contain infarcts, thromboses and cysts produced by hemorrhage. The follicles are atrophied and the pulp hypertrophied and hyperemic. The spleen is crowded with red corpuscles, but a few foci of extramedullary hematopoiesis containing nucleated red cells are often seen. Tuberculosis has been found in the spleen in a number of the patients,<sup>528</sup> including two of our own.

The liver is often enlarged and is strikingly hyperemic. Myeloid metaplasia may be found.<sup>436</sup> Cirrhosis may be discovered. Renal changes are absent, unless chronic renal disease has been associated, and there may be no cardiac hypertrophy. No remarkable changes are seen in the lymph nodes as a rule, although there may be deposits of hemosiderin, and hematopoietic foci have been observed.

The bone marrow has been described (page 994). It is red in the long bones as well as in the short and flat bones, but in the former it is engorged chiefly with mature erythrocytes, whereas in the short bones there is evidence of active hematopoiesis.<sup>345</sup>

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## *Methemoglobinemia and Other Disorders Usually Accompanied by Cyanosis*

### Introduction

#### Cyanosis

#### Oxidation of Hemoglobin

#### Methemoglobinemia

##### Acquired Methemoglobinemia

##### Hereditary Methemoglobinemia

##### NADH-Methemoglobin Reductase (Diaphorase) Deficiency

##### Other Metabolic Abnormalities Possibly Associated with Methemo- globinemia

##### Abnormal Hemoglobins, Hb M

#### Sulfhemoglobinemia

#### Carboxyhemoglobin. CO Poisoning

#### Detection of Abnormal Hemoglobin Pig- ments

## Introduction

### Cyanosis

The term "cyanosis" refers to a bluish color of the skin and mucous membranes. The color of these tissues depends on the pigment contained therein, the degree of dilatation or contraction of the blood vessels circulating in them, and the color of the fluid in the vessels. The color of the blood depends on the quantity and nature of the hemoglobin carried in the red blood corpuscles. A bluish color develops when there is an increase in the amount of reduced hemoglobin or of hemoglobin derivatives in the red cells.

Cyanosis usually is most marked in the lips, nail beds, ears, and malar eminences. Most commonly it is due to the presence of excessive amounts of reduced hemoglobin, much less often to the presence of other hemoglobin derivatives. As a general rule, cyanosis becomes apparent when the mean capillary concentration of reduced hemoglobin exceeds 5 g/dl. However, it is the absolute rather than the relative amount of reduced hemoglobin which is important in producing cyanosis. For this reason, if the same proportion of hemoglobin is reduced, cyanosis will be more evident when the total hemoglobin level is high, as in polycythemia, than when anemia exists.

In contrast to reduced hemoglobin, concentrations of only 1.5 to 2.0 g/dl of methemoglobin and as little as 0.5 g/dl of sulfhemoglobin will produce the degree of cyanosis associated with 5 g/dl of reduced hemoglobin at normal hemoglobin levels.

Decreased arterial oxygen saturation may result from impaired pulmonary or cardiac function, the presence of anatomic shunts (as in congenital heart disease), decreased atmospheric pressure, or alteration in the oxygen-combining capacity of the hemoglobin. The physiology of hemoglobin was discussed in Chapters 3 and 4 and the effects of decreased atmospheric pressure in producing polycythemia were considered in Chapter 30. In

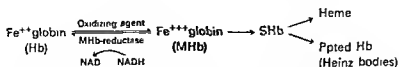


Fig 31-1 Steps in the oxidation of hemoglobin (simplified and schematic)  
SHb refers to sulfhemoglobin

the case of the hemoglobin derivatives, methemoglobin, sulfhemoglobin and carboxyhemoglobin, decreased oxygen saturation is due to the fact that these compounds are incapable of combining with oxygen.

Long-standing cyanosis dating from birth and unaccompanied by obvious cardiac and pulmonary disease was first reported by Francois in 1845.<sup>76</sup> Although it was appreciated that cyanosis could be induced by "blood poisons," it was 1891 before Dittrich<sup>76</sup> emphasized that methemoglobinemia tended to disappear spontaneously without any alteration in the concentration of circulating erythrocytes, thereby suggesting the existence of a reducing mechanism in erythrocytes. At the turn of the century, the concept of "auto-toxic enterogenous cyanosis," a disorder attributed to the formation of intracellular methemoglobin or sulfhemoglobin by toxic substances, possibly of bacterial origin absorbed from the gastrointestinal tract, was introduced. Ultimately it was recognized that methemoglobinemia could be observed in the absence of exposure to drugs, chemicals, or gastrointestinal disease, and by 1932 a familial incidence of idiopathic cyanosis was reported (Hitzenberger).<sup>76</sup> Hörlein and Weber, in 1948, described a German family in which hereditary methemoglobinemia appeared to be transmitted as a dominant characteristic.<sup>73</sup> The abnormality was shown to be in the globin moiety<sup>110</sup> and thus the first hemoglobinopathy was recognized. Subsequently, Scott and Hoskins<sup>104</sup> noted a high incidence of hereditary methemoglobinemia in Alaskan Eskimos and Indians, and Scott with Griffith,<sup>103</sup> in the following year, reported the absence of an enzyme, later called NADH-methemoglobin reductase (diaphorase), in the erythrocytes of these people.

"Enterogenous cyanosis" is no longer considered to be an entity. Cases reported as such can best be explained by analgesic drug ingestion. Patients ingesting commonly used analgesic and antipyretic drugs, such as acetanilid and phenacetin, which produce methemoglobinemia and sulfhemoglobinemia, not infrequently are neurotic and may deny drug use, but examination of the urine is likely to reveal metabolites of such drugs.<sup>36</sup> Yet the concept of enterogenous cyanosis persists. Experimental evidence has been reported that bacterial overgrowth in the intestine can cause oxidative damage to red cells. Such an effect could make erythrocytes more susceptible to the action of oxidant drugs.<sup>49</sup>

### Oxidation of Hemoglobin<sup>41</sup>

The iron of the heme moiety of hemoglobin has been considered to be in the ferrous state to permit reversible binding and transportation of oxygen. It has been assumed that, when hemoglobin is exposed to oxidants, the divalent (ferrous) iron is oxidized to the trivalent (ferric) state, forming methemoglobin. This concept for oxyhemoglobin has been challenged<sup>48</sup> and evidence which supports the view that the heme iron of oxyhemoglobin is in the ferric low spin state has been published.<sup>50</sup> The nonreactivity of the superoxide which is formed is preserved because of the location of the oxygen-binding site in a very hydrophobic region of the globin polypeptide chain (Chapter 4). Thus, methemoglobin may be formed by the dissociation,  $\text{HbO}_2 \rightarrow \text{MHb} + \text{O}_2^-$ , as distinguished from the commonly accepted manner described above and in Figure 31-1. This does not preclude other means of formation of methemoglobin, such as the direct



oxidation of the ferrous heme of deoxyhemoglobin by various drugs and chemicals.<sup>78</sup>

Methemoglobin can be converted to hemoglobin by reducing mechanisms in the erythrocyte (Chapter 3). Several metabolic pathways can be used for such conversion, but all of them are linked to the regeneration of reduced pyridine nucleotides. The major pathway involves a NADH-methemoglobin reductase. Another is thought to involve direct nonenzymic reduction of methemoglobin by reduced glutathione (GSH) when the hexose monophosphate shunt pathway (Chapter 3) is inactive.

Further oxidation of hemoglobin leads to the production of still other pigments. These are not well defined chemically, but all are forms of irreversibly denatured hemoglobin with heightened absorption of red light.<sup>21</sup> They include sulfhemoglobin, verdoglobin, and choleglobin. The exact nature of sulfhemoglobin remains to be determined. It is a brown to green hemichrome and is defined only by its solubility and its spectral absorption band at 620 nm, a band which does not disappear upon the addition of cyanide. Additional oxidation leads to rupture of the heme-globin linkage and intracellular precipitation of Heinz bodies, which in turn are removed by the spleen (Chapter 5). Thus, an overlapping series of reactions takes place as hemoglobin is oxidized.<sup>1</sup>

What factors determine the relative proportions of methemoglobin, sulfhemoglobin, and other compounds which are formed are not well understood. They include the nature of the oxidant; the intrinsic protective mechanisms of the red cell; whether or not these mechanisms are intact, waning as an effect of aging,<sup>21</sup> or absent as the result of genetic deficiency; and possibly altered as the effect of other factors, including the availability of sulfide.

## Methemoglobinemia<sup>20</sup>

As discussed in Chapter 3, methemoglobin formation occurs *in vivo* at a rate of about 3% a day. This is counterbalanced by a more rapid reduction process. However, under cer-

tain circumstances these mechanisms may be overcome.

### Acquired Methemoglobinemia<sup>5,14,41</sup>

Various chemical compounds used in the home or in industry, or as therapeutic agents, can cause methemoglobinemia (Table 31-1). These compounds or their breakdown products, if present in sufficient amounts, overcome the normal reducing mechanisms. Infants have been found to be especially susceptible to methemoglobinemia, probably because of transient deficiency of the enzyme, NADH-methemoglobin reductase (diaphorase). Perhaps other factors, such as a greater susceptibility of fetal hemoglobin to the action of nitrite as compared with that of adults, also may play a role.<sup>20</sup>

### Causes

A number of substances are capable of oxidizing hemoglobin directly and will do so *in vitro*. These include nitrites, nitrates, chlorates, and quinones. The nitrates, when ingested, are reduced to nitrites in the intestinal tract. Poisoning, sometimes fatal, has been reported a number of times, for example, as the result of the drinking, by infants, of well water high in nitrates<sup>6,25</sup> or of milk prepared by mixing the dried product with water containing nitrates. Nitrites may be absorbed following the use of bismuth subnitrate, ammonium or potassium nitrate, silver nitrate in the treatment of burns,<sup>10,39,45</sup> or the ingestion of foods high in nitrates or food adulterated with nitrites.<sup>28,30,37,43</sup> Methemoglobinemia also has been reported following renal dialysis performed in the home when nitrate-contaminated well water was used.<sup>7</sup>

An indirect effect is postulated in the case of certain aromatic amino and nitro compounds, including acetanilid (Bromo Seltzer), phenacetin (APC, Empirin, Anacin, Stanback), sulfonamides, and anilin dyes.<sup>5,14</sup> Most of these agents do not form methemoglobin from hemoglobin *in vitro* and are assumed to do so as the result of conversion to some extremely active intermediate compounds. The ingestion of a toy-shaped deodorant

Table 31-1. Methemoglobinemia

I Acquired Methemoglobinemia, causative agents		
Type of Compound	Therapeutic Agents	Domestic and Industrial
A Nitrites and other direct oxidants	Amyl nitrite	Food adulterated with nitrites
	Sodium nitrite	Corning extract
	Nitroglycerin	Nitrous gases (arc welders)
	Bismuth subnitrate	Well water (nitrates)
	Ammonium nitrate	Food high in nitrates
	Silver nitrate (burns)	Potassium chlorate
	Quinones	Anilin dyes
B Indirect oxidants	Sulfonamides	Diaper marking ink
Aromatic amino	Sulfanilamide	Dyed blankets
and nitro compounds	Prontosil	Laundry marks
	Sulfathiazole	Freshly dyed shoes
	Sulfapyridine	Red wax crayons
	Sulfamethizole	Naphthalene (moth balls)
		2 Anilinoethanol
	Misc aromatic compounds	
	Acetanilid	
	Phenacetin	
	Benzocaine (suppositories)	
	Prilocaine (local anesthetic)	
	In soap enemas	
	Phenylenediamine	
	Toluenediamine	
	Aminophenol	
	Nitrobenzenes	
	Trinitrotoluene	
	Resorcin	
II Hereditary		
A Enzymatic and other erythrocyte metabolic abnormalities		
1 NADH-methemoglobin reductase (diaphorase) deficiency (recessive)		
2 Other abnormalities?		
B Hemoglobin M disease (dominant) (See Table 31-2)		

containing naphthalene and aniline<sup>3</sup> or red wax crayons containing p-nitroaniline<sup>31</sup>; contact with marking ink,<sup>18</sup> dyed blankets, or laundry marks on diapers; and benzocaine,<sup>19</sup> prilocaine,<sup>41</sup> resorcin, anilin dyes, or other aromatic compounds absorbed by mouth, rectally,<sup>22</sup> or percutaneously<sup>41</sup> have led to methemoglobinemia. Likewise, excessive exposure to such compounds industrially has been reported to produce methemoglobinemia.<sup>4</sup> Exposure to naphthalene was associated with methemoglobinemia in G6PD-deficient infants,<sup>16</sup> and malaria prophylaxis has been reported as provoking methemoglobinemia in unsuspected heterozygotes deficient in NADH-methemoglobin reductase.<sup>8</sup>

### Pathogenesis and Symptomatology

The rapidity of methemoglobin production depends on (1) the extent and rate of entry of the compound into the individual's circulation and into the erythrocytes; (2) the metabolism of the offending chemical compounds within the body; (3) the extent to which the compound is converted to intermediates with either increased or decreased oxidizing capacities; (4) the excretion of the compound; and (5) the rate at which the erythrocytes can reduce methemoglobin to hemoglobin. Thus, the effect of nitrite introduced intravenously is expended within an hour, while nitrobenzene does not produce its maximum effect for 12 to 15

hours.<sup>9</sup> The extent to which these factors are influential in different persons possibly may vary and this may explain why some seem to be more likely to develop methemoglobinemia or sulfhemoglobinemia than others.

Symptoms vary in intensity but are often mild. Concentrations of 10 to 25% methemoglobin produce cyanosis but are tolerated without apparent ill effects; at 35 to 40%, slight exertional dyspnea and headaches, as well as fatigue, tachycardia, and dizziness, may be experienced.<sup>5,14</sup> Lethargy and stupor may appear with concentrations of about 60%; the lethal concentration probably is greater than 70%.<sup>20</sup> Only rarely is enough methemoglobin present to cause death. Nevertheless, the mortality in infants fed formulas prepared with well water containing high concentrations of nitrate has been as high as 10%. Heinz bodies may be formed in the red corpuscles and hemolytic anemia may occur also. The high concentrations (50% or higher) have been observed in toxic methemoglobinemia but not in the hereditary forms.

The signs of toxicity in acquired methemoglobinemias have been noted to be greater than those produced by a corresponding degree of anemia. Consequently, some further action besides the lowering of the oxygen-carrying power of the blood has been suspected. It had been assumed that the toxic symptoms not accounted for by the lowered amount of available oxyhemoglobin were due to the effect of the methemoglobin-producing agent on the tissues. It was found, however, that a definite and reversible increase in oxygen affinity occurs, as in the presence of carboxyhemoglobin.<sup>13</sup> This means that in methemoglobinemia the tissues are subjected to anoxemia, not only from loss of the oxygen capacity of the blood, but also from increased difficulty in unloading from the blood such oxygen as is available. This effect has been attributed to the formation of compounds intermediate between reduced hemoglobin, in which all four iron atoms are ferrous, and methemoglobin, in which all are ferric. The conversion of one or more of the four ferrous atoms in the hemoglobin molecule to ferric

may lead to an increased affinity of the remaining ferrous atoms for oxygen.

### Treatment

No therapy other than prohibition of the offending chemical agent is needed if the methemoglobinemia is sufficiently mild, since reduction of the methemoglobin will occur as the result of the intact, normal reversion mechanism. If symptoms are sufficiently pronounced, methylene blue, 1 mg/kg body weight in a 1% solution, slowly given iv over a period of five minutes, is the agent of choice. In infants twice this dose is used. To patients of any age, if cyanosis has not disappeared within an hour, a second dose of 2 mg/kg body weight should be given. Dosages should not exceed 7 mg/kg since toxic effects such as dyspnea, precordial pain, restlessness and apprehension, a sense of oppression, fibrillar tremors, and even persisting cyanosis and hemolytic anemia<sup>15</sup> can develop. Methylene blue may also be given orally in doses of 3 to 5 mg/kg. Ascorbic acid has been administered orally in doses of 100 to 500 mg/day,<sup>2</sup> but methylene blue is more effective since it brings about reversion of methemoglobin by activating the pentose phosphate cycle (page 102) rather than by nonenzymatic reduction, a process which is slower than the normal system of cell conversion.

### Hereditary Methemoglobinemias

The hereditary forms of methemoglobinemia are much rarer than the acquired disorder. An enzymatic form and several varieties due to the inheritance of certain abnormal hemoglobins are recognized.

#### *NADH (DPNH)-Methemoglobin Reductase (Diaphorase) Deficiency*

Since this form of hereditary methemoglobinemia was specifically identified,<sup>103</sup> at least 100 patients have been shown to be affected.<sup>78</sup> Before a specific metabolic defect in the erythrocyte had been recognized, a

number of patients with hereditary methemoglobinemia in whom methemoglobinemia responded to the administration of methylene blue or ascorbic acid had been reported.<sup>60</sup> If these patients are assumed to have had the same illness, about 260 examples of proved or assumed DPNH-methemoglobin reductase deficiency can be cited.<sup>78</sup> Most cases have been in persons of European stock.<sup>52</sup> Reports of a high frequency in Navajos<sup>53</sup> and the earlier observations in Alaska<sup>72</sup> raised anthropologic questions of common origins, but inbreeding probably is a better explanation. Cases have been reported in Hindu, Chinese, North African, Cuban, and Puerto Rican families.<sup>20,76</sup> Investigations of erythrocytes from such patients have failed to reveal other consistent metabolic abnormalities.<sup>20,78</sup> In homozygotes the enzyme is completely lacking.<sup>21,103</sup> The absence of methemoglobin in heterozygotes is explained by the fact that the reducing capacity of red corpuscles is about 250 times the oxidizing activity.<sup>102</sup>

The characteristic clinical feature is the presence of cyanosis in contrast to the total lack or minimal character of other symptoms and signs. The patients are "more blue than sick." There is no clubbing of the fingers or evidence of cardiopulmonary disease. The contrast is especially striking if the cyanosis is pronounced. The hue may be slate-gray, gray-brown, or violet. It is generalized over the whole body but is particularly noticeable in the lips, the mucous membranes of the mouth, the tongue, the palate, the nose, over the cheekbones, on the ears, and at the extremities of the fingers and toes, especially under the nails. The cyanosis often has been present from birth.

Most of the methemoglobin is found within a minor population of red cells, presumably the aging ones and resulting from a decline in the activity of ancillary reduction pathways.<sup>84</sup> The decreased oxygen capacity which results from the presence of 20 to 50% methemoglobin in the blood of patients with long-standing untreated methemoglobinemia is sometimes associated with a mild compensatory erythrocytosis<sup>70,77,103</sup> but this is inconstant. The life span of the erythrocytes

is not altered, nor is that of the patients.<sup>20</sup> Severe mental retardation has been found in about 12% of children and young adults with NADH-methemoglobin reductase deficiency.<sup>78</sup> Reduced numbers of nerve elements and retarded myelination have been described in a few instances.<sup>77</sup> However, no really satisfactory explanation has been offered; the presence of another genetic or prenatal environmental abnormality seems possible.

Rapid screening tests for fast detection of red cell NADH-diaphorase deficiency have been described. One requires the addition of nitrated blood to a reaction mixture freshly prepared from stable stock reagents.<sup>83</sup> It is based on disappearance of fluorescence due to NADH, a principle used in the detection of various other red cell enzyme deficiencies (page 773). Another involves the brown color that forms on the addition of ferricyanide to blood and its failure to change to red when NADH is added, as occurs normally.<sup>99</sup> Assay of the NADH-methemoglobin reductase enzyme system in a hemolysate is required to confirm the diagnosis.<sup>17</sup>

Multiple aberrations in the NADH-methemoglobin reductase of human erythrocytes exist, some with and some without functional consequences.<sup>62</sup> Eight or more different electrophoretic variants have now been recognized.<sup>54,78,101</sup> One of these ("Boston Fast") appears to be associated with only a minimal decrease in activity, but significant methemoglobinemia was observed in association with five of them (Boston Slow, Duarte, Princeton, Puerto Rico, California).<sup>75</sup> Because of such observations, it has been postulated that this disorder is due simply to a deficiency of the enzyme or to the synthesis of an abnormal enzyme protein with reduced activity. The heterogeneous pattern of methemoglobin accumulation in vivo may arise from accelerated inactivation of variant NADH-methemoglobin reductase during the life span of the red cell.<sup>63,101</sup>

Treatment usually is not required, except for cosmetic reasons. Methylene blue, as described earlier (page 1013), may be used. Daily oral doses of 100 to 300 mg will usu-

ally maintain the concentration of methemoglobin at about 10% or less. Less efficacious is ascorbic acid, 500 mg daily by mouth.

### Other Metabolic Abnormalities Possibly Associated with Methemoglobinemia

Deficiency of NADPH (TPNH)-methemoglobin reductase has been described, but this defect was not associated with methemoglobinemia.<sup>100</sup> This would be expected since the reducing mechanism via the hexose monophosphate shunt is of much lesser importance than the glycolytic route. In a patient with 19% methemoglobinemia, inadequate synthesis of glutathione (GSH), inherited as a dominant trait, was thought to result in impaired glyceraldehyde-3-phosphate dehydrogenase activity and insufficient reduction of NAD to NADH, with consequent methemoglobinemia.<sup>112</sup> (See Fig. 3-14, page 103.) However, methemoglobinemia has not been found in patients with considerably less GSH in their erythrocytes.<sup>98</sup> Slight increases in methemoglobin have been reported in patients with hereditary hemolytic disorders associated with deficiencies in the activities of certain enzymes,<sup>87</sup> but none of these reports provides a basis for recognizing any significant forms of methemoglobinemia attributable to metabolic abnormalities of the erythrocyte other than NADH-methemoglobin reductase deficiency.

### Abnormal Hemoglobins Associated with Cyanosis

Altered heme function is associated with certain abnormal hemoglobins and this results in cyanosis. These are of three kinds: (1) hemoglobins that are unstable as the result of amino acid substitutions that affect their tertiary structure and alter hemoglobin linkage ("unstable hemoglobins, Chapter 24); (2) hemoglobins with reduced oxygen affinity (Hb Kansas, Hb Seattle, Table 24-1); and (3) the M hemoglobins, discussed below.

Hemoglobin M is rare but it is the most important hemoglobinopathy in Japan, where one form (Iwate) had been described originally as "hereditary nigremia," a condition

Table 31-2. Hemoglobin M Diseases

Hb M Boston <sup>1</sup> ( $\alpha_2^{58\text{Gyr}} \beta_2$ ) <sup>85</sup>
Hb M Iwate <sup>†</sup> ( $\alpha_2^{87\text{Iyr}} \beta_2$ ) <sup>89</sup>
Hb M Saskatoon <sup>‡</sup> ( $\alpha_2\beta_2^{83\text{Trj}}$ ) <sup>85</sup>
Hb M Hyde Park <sup>§</sup> ( $\alpha_2\beta_2^{92\text{Trj}}$ ) <sup>86</sup>
Hb M Milwaukee-1 ( $\alpha_2\beta_2^{87\text{Trj}}$ ) <sup>85</sup>

<sup>1</sup>Identical to Hb M Kiskunhalas,<sup>72</sup> Osaka,<sup>108</sup>

Gothenburg

<sup>†</sup>Identical to Hb M Kankakee,<sup>79,89</sup> Oldenburg<sup>98</sup>

<sup>‡</sup>Identical to Hb M Emory, Kurume,<sup>105</sup> Chicago,<sup>82</sup>

Radom<sup>71</sup>

<sup>§</sup>Identical to Hb M Akita<sup>107</sup>

characterized by blackish-brown blood.<sup>105</sup> Five different Hb M's have been distinguished according to the nature of the amino acid substitution in the  $\alpha$ - or the  $\beta$ -chains (Table 31-2).

The enzyme systems for reducing methemoglobin function normally in the erythrocytes of individuals with Hb M, but they are unable to reduce methemoglobin M to hemoglobin M. In four of the Hb M variants the distal ( $\alpha 58$ ,  $\beta 63$ ) or proximal ( $\alpha 87$ ,  $\beta 92$ ) histidine of the  $\alpha$ - or  $\beta$ -chain is replaced by tyrosine. Under such circumstances the ferric iron atom forms a stable bond with the phenolate side chains of the tyrosine molecule, the usual bond with histidine not being formed.<sup>98a</sup> The absence of the latter leads to slight alterations in the tertiary protein structure of the abnormal chain and stabilization of the quaternary structure, thereby lowering the oxygen affinity of the normal subunits.<sup>98a</sup> In Hb M<sub>Milwaukee-1</sub> the substitution of a glutamic acid for a valine in the heme pocket of the  $\beta$ -chains has been shown to lead to ionic linkage between the  $\gamma$ -carboxyl group of glutamic acid and the ferric iron.<sup>86,95</sup>

In addition to the M hemoglobins listed in Table 31-2, four have been reported which have not yet been fully characterized and therefore their significance is uncertain (Hb M<sub>Arhus</sub><sup>71</sup> Hb M<sub>Leipzig</sub><sup>54</sup> Hb M<sub>Milwaukee-2</sub><sup>97</sup> Hb M<sub>Reserve</sub><sup>91</sup>). Two M hemoglobins, Hb Sydney<sup>58</sup> and Hb Freiburg,<sup>80</sup> are unstable and were discussed in Chapter 24.

Cyanosis is usually the only clinical manifestation, but a shortened erythrocyte survival was reported in an individual with

Hb M<sub>Milwaukee-2</sub><sup>97</sup> and in one with Hb M<sub>Chicago</sub><sup>82</sup>, hemolytic anemia was reported in a patient with Hb M<sub>Akita</sub><sup>107</sup> as well as in the one with Hb M<sub>Freiburg</sub><sup>55</sup>. Inheritance, as with other hemoglobinopathies, is dominant. In the heterozygote the prognosis is good, but the homozygous state probably is incompatible with life. In those forms in which the abnormality is situated in the  $\beta$ -chain, cyanosis may appear only after a lapse of three or four months when the  $\gamma$ -chains have been replaced by the abnormal  $\beta$ -chains. It is inferred that the rate of synthesis of Hb M chains is lower than that of normal chains since, in most affected individuals, only 25 to 30% of the total hemoglobin is in the oxidized state. The methemoglobin seems to be present in all the red corpuscles.<sup>85</sup> Hb A is the other major component, except in Hb M<sub>Milwaukee-2</sub> which was found to be associated with Hb E.<sup>97</sup>

Hb M can be distinguished from other forms of methemoglobinemia by the spectroscopic examination of an acid methemoglobin hemolysate. Instead of the normal absorption maxima at 502 and 632 nm, wavelengths lower than 632 nm are found,<sup>66</sup> and there also are alterations in the spectrum between 500 and 600 nm which are distinctive for some of the variants.<sup>20</sup> Quantification is best accomplished, however, by electrophoresis at neutral pH after the hemolysate has been oxidized by potassium ferricyanide.<sup>64</sup>

## Sulfhemoglobinemia

Sulfhemoglobin, as mentioned earlier (page 1011), has not been completely characterized. It is unable to carry oxygen and, unlike methemoglobin, cannot be converted into hemoglobin.

Most of the oxidant drugs listed in Table 31-1 can convert hemoglobin to sulfhemoglobin, but the most common offenders are acetanilid<sup>117,125</sup> and phenacetin. Concentrations of sulfhemoglobin as high as 10 g/dl may be found without the life of the patient being endangered. Other than cyanosis, which may be very pronounced, few

or no symptoms can be attributed to the altered pigment. Since many of the patients who develop sulfhemoglobinemia are neurotic, their headaches and constipation, which are common complaints, probably cannot be attributed to the sulfhemoglobinemia. Symptoms of bromide intoxication from the ingestion of Bromo Seltzer often have complicated the clinical picture.<sup>117</sup> In some patients, increased red cell destruction, anemia, and Heinz bodies have been observed.<sup>118,123</sup>

Once formed there is no way of removing sulfhemoglobin except by venesection, which is unnecessary. Treatment consists of interdiction of the offending agent.

Why some patients develop methemoglobinemia and others sulfhemoglobinemia is unknown. Since sulfhemoglobinemia is relatively uncommon in spite of the widespread use of drugs such as phenacetin, some factor peculiar to the individual other than drugs may be required for its development. For a long time, constipation was assumed to provide hydrogen sulfide. As an alternative explanation the red corpuscle itself has been suggested as the source of hydrogen sulfide. In some of the patients the concentration of GSH in the erythrocytes has been elevated.<sup>119</sup> Only one case of congenital sulfhemoglobinemia has been reported.<sup>122</sup>

## Carboxyhemoglobin. CO Poisoning

Carboxyhemoglobin is produced by the combination of hemoglobin and carbon monoxide (CO). Hemoglobin in the form of carboxyhemoglobin (Hb CO) is unavailable for the carriage of oxygen. Hb CO is a dissociable compound, but the affinity of CO for hemoglobin is 218 times that of oxygen.<sup>145</sup> Consequently, if present in high enough concentration, CO is asphyxiating. On the other hand, if oxygen is provided, preferably 95% O<sub>2</sub>, 5% CO<sub>2</sub>, if necessary by positive pressure face mask, CO is displaced, the concentration being halved in 40 minutes under conditions of adequate ventilation.

Carbon monoxide is produced during

hemoglobin degradation and arises from the heme moiety.<sup>133</sup> Measurement of the endogenous production of <sup>14</sup>CO can be used to determine the life span of the red corpuscles (page 216).<sup>140</sup> Endogenous production of CO may be a hazard to infants in respirators and to men in submersibles and space capsules, and may add to the risk of closed-circuit anesthesia.<sup>136</sup> Almost any flame or combustion device emits CO; coal gas, water gas,<sup>142</sup> and the exhaust of automobiles are well-known sources.<sup>131</sup> CO is both colorless and odorless and its effects are insidious. In low concentrations in the inspired air (0.05% for one hour), resulting in a blood saturation of 20%, a mild or throbbing headache may develop. Longer exposure or higher concentrations, resulting in a blood saturation of 30 to 50%, cause headache, irritability, confusion, dizziness, visual disturbances, nausea, vomiting, and fainting on exertion. Exposure for one hour to concentrations of 0.1% in the inspired air results in blood saturations of 50 to 80% Hb CO with coma, convulsions, respiratory failure, and death. If the atmospheric concentration is sufficiently high, saturation of the blood proceeds so rapidly that unconsciousness may occur suddenly and without warning symptoms.

The most characteristic sign of CO poisoning is the cherry-red color of the skin and mucous membranes. This is due to the bright-red color of Hb CO.

The toxic effects of CO are solely the consequence of anoxia, but this may cause tissue changes such as edema, small hemorrhages, and perivascular infiltration with focal necroses. Consequently, although the administration of oxygen relieves the immediate ill effects of carbon monoxide inhalation, there may be a residue of permanent damage, especially to the central nervous system and the heart.<sup>136</sup>

## Detection of Abnormal Hemoglobin Pigments

If present in high enough concentrations, abnormal hemoglobin pigments can be de-

tected and the various types differentiated on gross examination of the blood. When they are present in lower concentrations, and for specific identification and quantitation, spectroscopic examination is necessary. In addition, appropriate studies should be made to demonstrate or rule out the presence of erythrocytosis, anemia, Heinz bodies (page 736), hemoglobinuria, and evidence of increased blood destruction (page 725). As discussed earlier (page 1016), starch block electrophoresis of hemolysates and spectrophotometric absorption analysis are required to establish a diagnosis of one of the Hb M disorders.

**GROSS EXAMINATION.** A sample of the blood, collected in a vessel containing an anticoagulant, should be centrifuged so that the plasma may be inspected for evidence of abnormal pigments and for hemolysis. The whole blood should also be inspected and then shaken in air for 15 minutes. Normally, the blood will become bright red as the reduced hemoglobin is converted to oxyhemoglobin. If it remains dark, abnormal intracellular pigments must be present. Blood containing methemoglobin is chocolate-brown. Sulfhemoglobin produces a mauve-lavender color. These colors are difficult to detect in whole blood, but, if dilutions of  $\frac{1}{100}$  or greater are made and the diluted blood is held against a white background, the colors are more easily differentiated. When carboxyhemoglobin is present, the blood is cherry-red. If there is uncertainty, 5 ml of 40% sodium hydroxide may be added to 5 ml of a 5% solution of blood in water. An oxyhemoglobin solution will turn brown, but a carboxyhemoglobin solution remains red.

**SPECTROSCOPIC AND OTHER METHODS OF EXAMINATION.** A hand spectroscope suffices if the concentration of the abnormal pigment is relatively high. If it is less than 10% or if quantitative measurements are required, it is necessary to resort to spectrophotometric<sup>24,152,169</sup> or colorimetric<sup>153</sup> and gasometric<sup>170</sup> procedures.<sup>185</sup>

The collection of the blood specimens

must be carried out with special care and should be prompt since, with the exception of sulfhemoglobin, the abnormal pigments disappear rapidly on removal of the causative agent or on institution of therapy. In obtaining the specimen, every precaution must be taken to prevent hemolysis. If hemoglobinemia is suspected, part of the blood should be allowed to clot. After allowing this portion to stand for an hour in the refrigerator, it is centrifuged without dislodging the clot. If carbon monoxide exposure is suspected, the blood should be collected in small tubes which are tightly stoppered, dry sodium citrate being used as an anticoagulant. For most other purposes, dry oxalate is preferable to heparin as an anticoagulant, but the latter is preferable if methemoglobin is suspected since oxalate increases the pH of the blood and favors the conversion of neutral methemoglobin to alkaline methemoglobin.<sup>165</sup>

In examining for methemoglobin and sulfhemoglobin, whole blood or washed red corpuscles are added to distilled water in a ratio of 1:10 or 1:100, depending upon the concentration of the abnormal pigment. A few ml of the hemolysed blood are placed in each of two test tubes. The first tube is then examined with the hand spectroscope. Methemoglobin produces a dark band at 630 nm in the red region of the spectrum (Plate XIII). To the second tube, 2 or 3 drops of 5% solution of potassium cyanide are added. If the pigment is methemoglobin, the band will disappear. Sulfhemoglobin produces a dark band at 620 nm which is difficult to distinguish from that of methemoglobin in the hand spectroscope, but it can be recognized by the fact that it is not removed by the addition of cyanide. Hydrogen peroxide (3%) causes both of these bands to disappear.

Spectrophotometry offers more precise as well as quantitative information. The essential principle<sup>151</sup> consists in the determination of the optical densities, at various wavelengths, of a dilute solution of hemolysed normal blood and repetition of the same procedure on the suspected sample. Details are given by Dubowski.<sup>152</sup>

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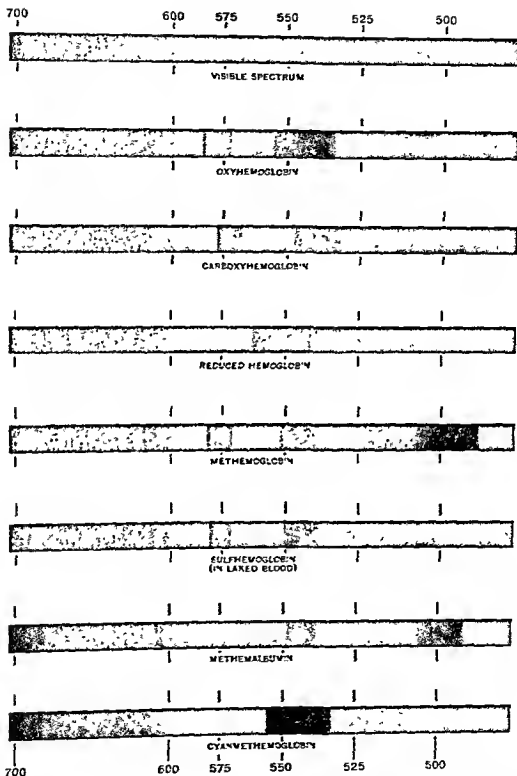
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# PLATE XIII

## SPECTRUMS OF HEMOGLOBIN



Spectrums of hemoglobin (From Levinson and MacFate, *Clinical Laboratory Diagnosis*, 1969 (Prepared by Captain V. E. Martens, MC, USN U S Naval Medical School, Bethesda, Maryland) The numbers indicate the wave lengths in nanometers (nm)

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## *The Porphyrrias*

Congenital Erythropoietic Porphyrria  
Acute Intermittent Porphyrria  
Variegate Porphyrria  
Hereditary Coproporphyrria  
Protoporphyrria  
Acquired Porphyrria

**T**HE porphyrias are a group of disorders characterized by the excessive production and excretion of porphyrins and porphyrin precursors. They result from hereditary or acquired defects in the pathways of heme biosynthesis (Chapter 4, page 169), and, for this reason, are often included within the discipline of hematology, even though abnormalities in the formed elements of the blood usually are absent or incidental.

At least six different kinds of porphyrias have been distinguished. These differ from one another clinically (Table 32-1) and in the type of porphyrin or pyrrole excreted (Fig. 32-1), the route of excretion, the major endogenous tissue source of the porphyrin or pyrrole, and the pattern of inheritance (Table 32-2).

### **Congenital Erythropoietic Porphyrria**

This rare inborn error of metabolism is characterized by the production in red cell precursors of porphyrins of isomer type I,

especially uroporphyrin I. Although probably first recognized by Schultz<sup>21</sup> and by Baumstark<sup>3</sup> in 1874, congenital erythropoietic porphyria was comprehensively described and distinguished from other porphyrias by Gunther in 1911.<sup>7</sup> It has been suggested that because their disease caused them to have red teeth and to be disfigured, hirsute, and nocturnal, persons with congenital erythropoietic porphyria may have been responsible for the werewolf legend.<sup>9</sup>

### **Genetics and Prevalence**

Congenital erythropoietic porphyria is inherited as an autosomal recessive trait and is by far the least common of the porphyrias, having been reported in only about 70 persons.<sup>17,20</sup> It occurs to a similar degree in males and females and has been described in a wide variety of racial groups, including English, French, Spanish, Italian, German, Polish and Norwegian Caucasians as well as Japanese and Indian Orientals and Bantus. Usually the illness is first detected in infancy and only rarely later in life.<sup>13</sup>

### **Clinical Description**

Often, the first sign of the disease is discoloration of the child's diapers by the urine, which ranges from pink to deep burgundy in color. The most prominent manifestation is pronounced cutaneous photosensitivity

Table 32-1. Major Clinical Manifestations of the Porphyrrias

Type of Porphyrin	Photosensitive Skin Lesions	Recurrent Acute Episodes*	Urine Appearance	Other Features
Congenital erythropoietic	Very severe	0	Red	Hemolytic anemia Erythrodontia
Acute intermittent	None	++	Darkens after standing	
Variegate	Moderately severe	+	Normal or red	
Coproporphyrria	Unusual	+	Normal or red	
Protoporphyrria	Mild	0	Normal	Biliary and hepatic disease
Acquired	Moderately severe	0	Red	Hepatic disease

\*Abdominal pain, peripheral neuropathy and mental symptoms often precipitated by drugs

(*hydraea aestivale*). Exposure to sun is followed by the development of vesicular or bullous lesions containing a porphyrin-rich fluid. The lesions tend to heal slowly, leaving pigmented scars. Often they become infected, ulcerated, and necrotic, leading, over a period of years, to progressive mutilation and disfigurement with loss of portions of the fingers, nose, eyelids, or ears (Fig. 32-2). Skin not exposed to light is unaffected. Often the patients adopt extreme precautions to avoid the sun.

Hypertrichosis is frequently present, usually manifested by downy, lanugo-like hair covering exposed parts of the body.<sup>16</sup> Deposition of porphyrin in the dentin of the teeth causes them to appear bright red (erythrodontia), brown, or yellowish. Even if discoloration is not apparent in ordinary light, the teeth may exhibit red fluorescence in ultraviolet light. If the ultraviolet light source is sufficiently intense, red fluorescence also may be seen in the phalangeal bones.

Hemolytic anemia is detected in the majority of the patients, and the spleen is almost always enlarged.

### Laboratory Findings

The anemia is normocytic and normochromic and tends to be mild in degree. Severe anemia requiring blood transfusions<sup>5</sup> is unusual. The anemia results from excessive red cell destruction, but the intensity of hemolysis varies considerably from one patient to another and in the same patient at different times. When moderate to severe, it is accompanied by reticulocytosis, erythroid hyperplasia of the marrow, increased excretion of bile pigments, and reduced values for erythrocyte life span.<sup>17,20</sup> Ineffective erythropoiesis may be prominent, as manifested by a pronounced increase in "early-labeled" bile pigments (Chapter 5).<sup>15</sup>

The most characteristic metabolic abnormality is greatly increased urinary excretion of uroporphyrin I, a biologically useless isomer that cannot be converted to heme. Increased urinary excretion of uroporphyrin III<sup>24</sup> and coproporphyrin I may also occur, but to a lesser extent than that of uroporphyrin I. Trace quantities of porphyrins with 7, 6, 5, and 3 carboxyl groups also may be

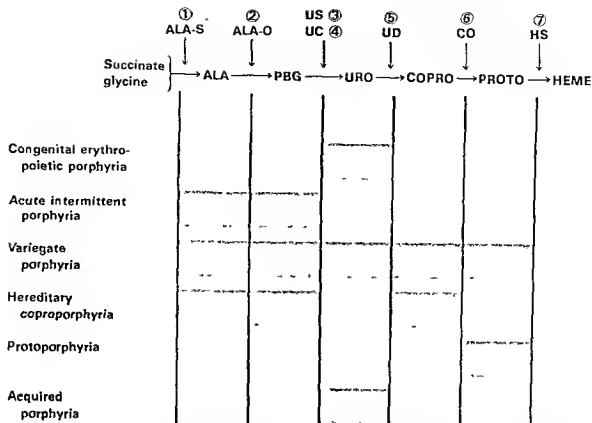


Fig 32-1. Patterns of porphyrin and precursor excretion in six types of porphyria. The pathway of heme biosynthesis is shown at the top of the figure. The major intermediates excreted in each type of porphyria are indicated by the shadow areas below the appropriate intermediate. ALA = delta aminolevulinic acid. PBG = porphobilinogen. URO = uroporphyrin. COPRO = coproporphyrin. PROTO = protoporphyrin. Each of the biosynthetic enzymes is numbered: (1) ALA synthetase (ALA-S), (2) ALA dehydratase (ALA-O), (3) uroporphyrinogen I synthetase (US), (4) uroporphyrinogen III cosynthetase (UC), (5) uroporphyrinogen decarboxylase (UD), (6) coproporphyrinogen oxidase (CO), (7) heme synthetase (HS).

detected in the urine.<sup>18</sup> Fecal excretion of porphyrins, especially coproporphyrin I, is increased<sup>1,2,12</sup>; uroporphyrin I is greatly increased in erythrocytes<sup>1,20</sup> and in plasma.<sup>1</sup>

#### Nature of the Metabolic Abnormality

Studies with fluorescence microscopy suggest that the excess porphyrins are formed in red cell precursors.<sup>20,23</sup> When bone marrow is examined in this way, there appear to be two populations of normoblasts, one normal and one in which excessive amounts of porphyrin are present, especially in the area about the nucleus. The abnormal population is further characterized by nuclear inclusions,<sup>6,9</sup> probably containing hemoglobin,<sup>20</sup> and by excessive cytoplasmic iron granules.<sup>23</sup>

Studies of the porphyrin biosynthetic pathway in erythrocytes, as well as in skin fibroblasts, show that the activity of uroporphyrinogen III cosynthetase (uroporphyrinogen isomerase) is severely impaired (Fig. 32-1, reaction 4).<sup>19</sup> Furthermore, levels of this enzyme in heterozygotes are intermediate between those present in homozygous patients and those in normal subjects. It is reasonable to conclude that lack of this enzyme constitutes the primary genetic abnormality; as a result, much of the porphobilinogen normally utilized for uroporphyrinogen III synthesis is converted instead to uroporphyrinogen I, a compound which, being unutilizable, must be excreted. However, this formulation would not easily account for the occasionally observed increase in uroporphyrin III excre-

Table 32-2. Genetic and Metabolic Abnormalities in the Porphyrrias

Type of Porphyrria	Heredity	Probable Abnormality	Porphyrins or Pymoles Excreted*	Route of Excretion*	Tissue Source
Congenital <u>erythropoietic</u>	<u>Autosomal recessive</u>	Decreased uroporphyrinogen III cosynthetase	<b>Uroporphyrin I</b> Uroporphyrin III Coproporphyrin I 7, 6, 5 and 3 COOH porphyrins	Urine	Erythropoietic
Acute intermittent	Autosomal dominant	Decreased uroporphyrinogen III synthetase	<b>Porphobilinogen</b> † δ-Aminolevulinic acid†	Urine	Hepatic
Variegate	Autosomal dominant	Increased ALA synthetase	<b>Coproporphyrin</b> } <b>Protoporphyrin</b> } Porphobilinogen† δ-Aminolevulinic acid†	Feces Urine	Hepatic
Coproporphyrria	Autosomal dominant	Unknown	<b>Coproporphyrin</b> } <b>Porphobilinogen</b> † δ-Aminolevulinic acid†	Feces Urine	Hepatic
Protoporphyrria	Autosomal dominant	Unknown	<b>Protoporphyrin</b>	Feces	Erythropoietic and/or hepatic
Acquired <i>Porphyrria cutanea tarda</i>	Acquired	Decreased uroporphyrinogen III synthetase	<b>Uroporphyrin I</b>	Urine	Hepatic

\*The major metabolite or route is shown in boldface

†May be absent during remissions

‡Usually only during acute attacks



Fig. 32-2 Congenital erythropoietic porphyria in an Indian boy. Note facial hirsutism, scarring, and discoloration of the teeth.

tion nor for the lack of an overall deficiency of heme production. An alternative, less plausible hypothesis holds that the primary genetic defect leads to production of δ-aminolevulinic acid (Fig. 32-1, reaction 1) in such large amounts that the capacity of subsequent enzymes to convert it to uroporphyrinogen III is exceeded.<sup>24</sup>

### Treatment

Splenectomy may partially or completely relieve the hemolytic anemia<sup>3</sup> and may also lead to reduced porphyrinuria and photosensitivity.<sup>1,23</sup> Furthermore, hypertransfusion to suppress erythropoiesis decreases porphyrin excretion.<sup>8</sup> However, splenectomy is not always necessary and hypertransfusion is not a practical long-term therapeutic device. Most patients rely on avoidance of exposure to sunlight. They should be instructed to wear special protective clothing, including gloves and broad-brimmed hats, to cover all

possible cutaneous surfaces. Conventional sun-screening agents and sunburn preventatives are ineffective since they screen out wavelengths less than 300 nm. The active wavelength with respect to porphyrins is about 400 nm,<sup>16</sup> which is near the visible range. Thus, to be effective, a local agent must be visible on the skin. Reflective materials such as zinc oxide or titanium dioxide are useful, as is ordinary pigmented theatrical makeup.<sup>16</sup> Preparations containing substituted quinones (eg, lawsone) and dihydroxyacetone turn the skin a cosmetically acceptable brownish-tan and may also be effective.<sup>4,22</sup>

### Congenital Porphyria in Animals

Congenital porphyria ("pink-tooth") in cattle appears to be very similar if not identical to the human disorder. In cattle, the disease is inherited as an autosomal recessive trait. Photosensitivity of skin areas not covered by pigmented hair as well as hemolytic anemia have been observed. The teeth and bones are stained red. Uroporphyrin I and smaller amounts of coproporphyrin I are excreted in the urine. Furthermore, uroporphyrinogen cosynthetase activity is severely impaired in erythrocytes.<sup>19</sup> A similar disorder has been observed in pigs, but the inheritance pattern appears to be dominant.<sup>10</sup> In the fox squirrel (*Sciurus niger*), little uroporphyrinogen III synthetase is found, and the excretion and tissue deposition of uroporphyrin I are similar to those in congenital erythropoietic porphyria, but this species seems to suffer no ill effects.<sup>14</sup>

## Acute Intermittent Porphyria

### (Pyrroloporphyria, Swedish Porphyria)

Acute intermittent porphyria is a disorder in which delta-aminolevulinic acid (ALA) and porphobilinogen (PBG) are produced in excess by the liver and are excreted in the urine. This metabolic abnormality is accompanied by acute attacks of mental, abdominal, and neurologic symptoms. Although patients

with acute intermittent porphyria probably were reported by Gunther,<sup>7</sup> it was the extensive family studies made in Sweden by Waldenstrom that provided the first comprehensive descriptions.<sup>71</sup>

### Genetics and Prevalence

Inherited as an autosomal dominant trait,<sup>22,67,72</sup> this type of porphyria is relatively common. The overall prevalence has been estimated at 1.5 per 100,000 population,<sup>44</sup> but in some areas a much greater prevalence has been noted, for example, in Lapland (1:1000)<sup>44</sup> and in Sweden (1:13,000).<sup>76</sup> Not only is this disorder more common in women (60 to 75% of patients) than in men, but the frequency and severity of attacks are also considerably greater in women.<sup>22,67,72</sup> The onset of the disease usually occurs in late puberty or early adulthood, but may occur later in men (average age, 36 years) than in women (average age, 25 years).<sup>67</sup>

### Clinical Description

The clinical course is marked by recurrent, acute attacks lasting several days to several months. These are interspersed with long and variable asymptomatic or latent periods. There may be as few as three attacks in a lifetime or as many as two or three per year.<sup>67</sup> Acute attacks may be precipitated by exposure to certain drugs, barbiturates and sulfonamides being the most common but many others have also been implicated (Table 32-3). In some women, attacks occur just prior to the menstrual period. Other precipitating factors include decreased food intake,<sup>53,77</sup> infection, and alcohol excess. In about 25% of attacks, the precipitating factors are unknown.<sup>67</sup> Endogenous steroid metabolites having the 5 $\beta$ -H configuration may play a role in precipitating some of these attacks.<sup>46,47,52</sup>

Acute porphyric episodes are characterized by a triad of abdominal, neurologic, and mental symptoms. The most common one is abdominal pain, occurring in about 95% of



**Table 32-3. Drugs and Toxins Which May Precipitate Acute Attacks in Acute Intermittent Porphyrria, Variegata Porphyrria, or Coproporphyrria<sup>22,36</sup>**

<i>Evidence for Porphyrrogenic Effect</i>		
<i>Class of Drug</i>	<i>Case Reports (in Patients)</i>	<i>Animal Studies or Liver Cell Culture</i>
Sedatives	Barbiturates	Glutethimide (Doriden)
	Meprobamate	Methyprylon (Noludar)
	Sedormid	Librium
	Sulfonal	Carbromal
	Tonal	Ethinamate (Valmid)
	Chloral derivatives	Hydroxydione
Antibacterial and antifungal agents	Sulfonamides	Chloramphenicol
	Griseofulvin	Isoniazid Pyrazinamide
Hypoglycemic agents	Tolbutamide	
Anticonvulsants	Dilantin	Tridione
	Celontin	
	Milontin	
	Mesantoin	
Steroids	Progesterone	Testosterone
	Estrogens	5 $\beta$ -H steroids
	Contraceptive pills	Metyrone
Anelgesics	Aminopyrine	Probenecid
CNS stimulants		Nikethamide
		Bemegride
Antihypertensives	Methyl dopa	
Toxins	Lead	Hexachlorobenzene
	Alcohol	Lindene
	Arsenic	Chlordene
		Allyl isopropyl ecetyl carbamide (AIA)*
		Diethyl dihydro collidine (DDC)*

\*Agents used to induce experimental porphyrria in animals

the patients.<sup>67</sup> The pain is usually described as moderate to severe in degree and as crampy or colicky in nature. It may be generalized or periumbilical, or located predominantly in the lower quadrants. It is characteristically accompanied by constipation and, often, by vomiting. Because these symptoms have mistakenly been attributed to a variety of conditions requiring emergency operations, many of the patients have been subjected to unnecessary laparotomy. Dilated bowel loops may be palpable, but the abdo-

men usually is soft, and there is no rebound tenderness or other sign of peritoneal irritation.

The patients often complain of pain or paresthesias in the extremities, but motor symptoms usually are the dominant sign of peripheral neuropathy. There may be mild to moderate weakness in one or more extremities and these manifestations may progress to flaccid quadriplegia. In one series, 11 of 35 patients developed quadriplegia and 10 others had less generalized motor symp-

toms.<sup>67</sup> Paralysis of respiration, the most serious manifestation of the neuropathy, is less common. It may be so severe that mechanical assistance is required.<sup>42</sup> Other relatively less common neurologic manifestations include the "restless legs" syndrome,<sup>49</sup> and impaired function of the facial, extraocular, trigeminal, and other cranial nerves. Deep tendon reflexes commonly are absent. Frequently there are tachycardia,<sup>64</sup> hypertension, postural hypotension, inappropriate sweating, and bladder distension, findings which are thought to be manifestations of autonomic neuropathy.

Mental symptoms resemble a toxic delirium and include hallucinations, confusion, and acute anxiety states.<sup>33,61</sup> Some of these symptoms may be a manifestation of hyponatremia (page 1028). Occasionally, manic-depressive or schizophrenic behavior is observed.<sup>22,33</sup> Tonic-clonic seizures were observed in 20% of patients in one series.<sup>67</sup> Depression, including suicidal tendencies, may follow an attack.

### Laboratory Findings

Results of routine hematologic measurements usually are within normal limits; however, leukocytosis occasionally is observed during acute attacks,<sup>67</sup> and this finding may support an erroneous impression of an abdominal condition requiring an emergency operation. In one series, four patients with iron-deficiency anemia and two with unexplained, normocytic, normochromic anemia were found among 46 patients with the disease.<sup>67</sup> More frequently, the red cell mass is reduced even though the VPRC is normal.<sup>31</sup> Red cell survival is normal, suggesting that the decreased red cell mass results from reduced effective erythropoiesis.<sup>31</sup>

The characteristic abnormality of porphyrin metabolism is the excessive urinary excretion of PBG and, to a lesser extent, ALA. These porphyrin precursors can be quantified accurately by means of a chromatographic method.<sup>56</sup> Normally, less than 3 mg of either ALA or PBG are excreted in 24 hours. In

21 symptomatic patients, an average of 83 mg of PBG per 24 hours (range 30 to 200) and 43 mg of ALA per 24 hours (range 8 to 150) were excreted.<sup>67</sup> ALA and PBG excretion tends to decrease somewhat during remission. In 12 asymptomatic patients, the average urinary PBG was 32 mg/24 hours (range 12 to 60) and ALA was 10 mg/24 hours (range 6 to 18).<sup>67</sup> In exceptional patients, values fall into the normal range during latent periods.<sup>22,55</sup>

Urinary PBG also may be detected by a qualitative method, the Watson-Schwartz test.<sup>72</sup> This test yields negative results in normal subjects and positive ones when PBG exceeds about 6 to 8 mg/24 hours.<sup>22</sup> When properly performed and interpreted, the Watson-Schwartz test may be relied upon. However, in routine laboratories, falsely positive results are common. In the experience of one referral center, 75% of patients with supposedly positive findings had normal values when the chromatographic method was used.<sup>67</sup> Urobilinogen, indoles, and certain indicator dyes such as pyridium, methyl red, skatol red, and melanogens may be excreted in the urine and confused with PBG in the Watson-Schwartz test. The precautions necessary to ensure proper interpretation of the results of the test have been outlined by Watson and his co-workers.<sup>22,74</sup>

Because PBG is a colorless substance, freshly excreted urine from patients with acute intermittent porphyria often appears normal in color. On exposure to light and air, especially at alkaline pH, PBG-containing urine darkens. The phenomenon is largely accounted for by the formation of porphobilin, a dark-brown, non-porphyrin oxidation product of PBG.<sup>73</sup> In addition, PBG may be converted nonenzymatically to a mixture of porphyrins, especially uroporphyrins.<sup>34</sup> Thus, under certain conditions, urine porphyrins may appear to be increased in persons with acute intermittent porphyria, but this finding is largely artifactual.

Fecal porphyrins may be slightly to moderately increased, but not to the degree found in association with variegate porphyria or coproporphyria.<sup>17,35,48</sup>

Hyponatremia, reduced blood volume, and mild elevation of blood urea nitrogen frequently are observed during acute attacks.<sup>67</sup> Often these findings can be attributed to sodium loss by vomiting. However, in a number of instances, the syndrome of inappropriate antidiuretic hormone secretion has been documented,<sup>50,54,67</sup> presumably owing to hypothalamic involvement. When carefully searched for, other evidence of hypothalamic abnormality has been found, such as disturbed regulation of growth hormone and ACTH secretion.<sup>62,75</sup> The serum protein-bound iodine often is increased, but this usually results from elevated levels of thyroxine-binding globulin rather than from hyperthyroidism.<sup>67</sup>

The results of liver function tests usually are within normal limits except for those of the Bromsulphalein (BSP) retention test. More than 10% of administered BSP was retained in 79% of symptomatic patients and in 55% of those in remission.<sup>67</sup> This abnormality appears to be due to a functional defect in the conjugation and excretion of BSP<sup>66</sup> and is not accompanied by structural abnormalities as shown by liver biopsy.<sup>51</sup>

Abnormal electroencephalographic (EEG) patterns are the most common laboratory signs of neurologic disease. Diffuse, nonspecific slowing of the wave pattern was found in 14 of 24 patients during an acute attack.<sup>67</sup> This abnormality tended to disappear when remission occurred. Less commonly, focal EEG abnormalities may be found in the absence of local neurologic disease. The cerebrospinal fluid usually is normal, but the protein concentration occasionally is slightly increased. In patients with neuropathy, the electromyogram may demonstrate a denervation pattern and denervation atrophy may be found on muscle biopsy.

Abdominal x rays may demonstrate distended bowel loops in patients with abdominal pain. Impaired glucose utilization is common during acute attacks; the fasting blood sugar value was usually normal, but the results of the glucose tolerance test were abnormal in 11 of 15 patients.<sup>67</sup> Hypercholesterolemia is common.<sup>69</sup>

## Nature of the Metabolic Abnormality

The activity of ALA synthetase (Fig. 32-1, reaction 1) is distinctly increased in liver tissue from patients with acute intermittent porphyria.<sup>70</sup> This observation led to the suggestion that the primary genetic defect is a constitutive operator mutation leading to overproduction of this enzyme. Although such a defect could account for excessive production of ALA and PBG, it does not explain why these precursors are not converted to porphyrins. In normal subjects, a significant fraction of administered ALA is excreted as porphyrins.<sup>37</sup> Furthermore, in patients with variegate porphyria (page 1029) an increase in ALA synthetase activity leads to excretion of several porphyrins in addition to ALA and PBG (Fig. 32-1). Accordingly, it has been suggested that the increased ALA synthetase found in patients with acute intermittent porphyria is a compensatory phenomenon, occurring as a result of a block elsewhere in the heme biosynthetic pathway. Such an increase would be consistent with a reduced effect of the known feedback inhibition and repression control exerted by heme on ALA synthetase activity (page 169). A logical site for the primary block in acute intermittent porphyria is at the step catalyzed by the enzyme uroporphyrinogen synthetase (Fig. 32-1, reaction 3), in which porphobilinogen is converted to porphyrins. Indeed, reduced levels of this enzyme were demonstrated in the liver<sup>58,63</sup> and erythrocytes<sup>57</sup> of patients with acute intermittent porphyria. Furthermore, subjects with latent disease could be detected by assay of this enzyme at a time when urinary excretion of ALA and PBG was normal.<sup>37</sup> Thus, reduced uroporphyrinogen synthetase activity, inherited as an autosomal dominant, appears to be the most likely primary genetic defect in this form of porphyria. The increase in ALA production may serve a compensatory function, making possible the synthesis of heme at a normal rate. Exogenous or endogenous factors which lead to further increases in ALA production, however, may precipitate acute attacks. These factors include drugs

(Table 32-3) as well as certain steroid metabolites.<sup>46,47,52</sup> The influence of the latter may account for the typical onset of the disease at puberty as well as the relation of acute attacks to events in the menstrual cycle of some women.

The relation between the metabolic abnormality and the symptoms remains a mystery. It seems reasonable to propose that PBG or a derivative is toxic to the nervous system. Indeed, PBG and porphobilin have been shown to inhibit neuromuscular transmission under experimental conditions.<sup>39</sup> On the other hand, it has not been possible to induce neurologic abnormalities by administering PBG to animals,<sup>43</sup> normal human subjects,<sup>37</sup> or even patients with latent porphyria.<sup>57</sup> Furthermore, there is at best only a partial correlation between the neurologic symptoms and urinary or plasma PBG levels.<sup>53</sup>

### Treatment and Prognosis

Prevention of acute attacks by avoiding exposure to toxins, especially barbiturates, is most important. Patients should be given a list of drugs to avoid (Table 32-3) and should carry it with them at all times. A high-protein, high-carbohydrate, low-fat diet should be maintained,<sup>22</sup> and the patients should be warned that fasting may induce attacks.<sup>53</sup> They should also know that maintenance of the diet is important enough to require the attention of a physician whenever adequate oral intake is interfered with by intercurrent disease. In women whose attacks are related to the menstrual cycle, prevention can be achieved by suppression of ovulation with androgens or estrogen-progesterone contraceptive pills.<sup>61,67</sup>

When an acute attack occurs, hospitalization usually is required. A primary goal of therapy, based on the repression of ALA synthetase by glucose,<sup>77</sup> is to administer calories in the form of carbohydrate and protein. At least 300 g of glucose should be given in the first 24 hours. If oral intake is possible, glucose may be given in fruit juice by mouth. If necessary, 10% glucose or fructose solutions may be administered intravenously.<sup>22</sup> In

patients with carbohydrate intolerance, insulin may be required.<sup>22</sup> With high carbohydrate administration, a pronounced decrease in urinary porphobilinogen was observed in 13 of 16 patients, and the acute attack was aborted in 10 of 14 patients with symptoms.<sup>67</sup> However, these data must be interpreted with caution, for spontaneous recovery may occur, and, as yet, carefully controlled studies have not been possible.

Phenothiazines are useful in symptomatic therapy.<sup>22,59</sup> Chlorpromazine may be given in doses of 25 mg or more four times a day. This may control pain as well as mental symptoms. Opiates may be employed if pain is severe. Lithium carbonate may be useful in treating depression.<sup>67</sup>

Preparation for artificial support of respiration should be made if neuropathy is progressive. Adequate respiratory care in the paralyzed patient can be life-saving.

Possible therapeutic benefit in acute intermittent porphyria has been attributed to adenosine monophosphate,<sup>41</sup> chelating agents,<sup>63</sup> zinc,<sup>65</sup> hematin,<sup>32</sup> vitamin E,<sup>60</sup> and deoxypyridoxine.<sup>38</sup> None of these modes of therapy has become established, and further investigation is required before their use can be recommended.

The acute attack of porphyria may be fatal. A mortality rate of 24% over a five-year period was reported in one series of 50 patients.<sup>43</sup> In another, more recent series, four fatalities were recorded among 46 patients.<sup>67</sup> Death usually results from the consequences of respiratory paralysis or from cardiac arrhythmias secondary to sympathetic discharge. The mortality rate appears to be decreasing,<sup>72</sup> probably because of earlier case-finding coupled with the prohibition of barbiturates and other toxic drugs.

### Variegate Porphyria

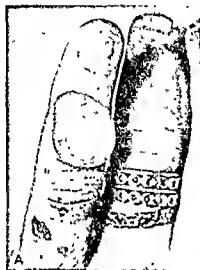
(Mixed Porphyria, Protocoproporphyria, Porphyria Cutanea Tarda Hereditaria, South African Genetic Porphyria)

Variegate porphyria is a disorder in which large amounts of protoporphyrin and copro-

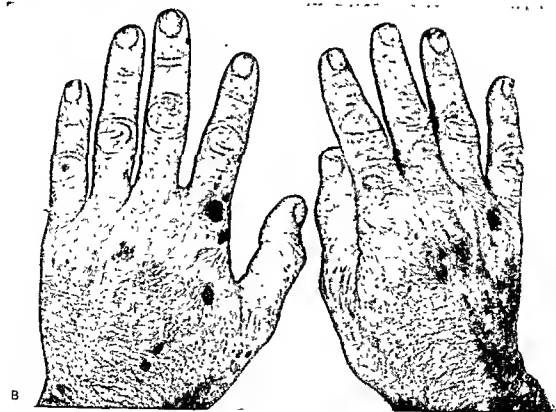
porphyrin are excreted in the feces. Like acute intermittent porphyria, the disease is marked by the occurrence of acute attacks that are accompanied by excessive urinary excretion of PBG and ALA and are often precipitated by ingestion of barbiturates. In

addition, however, variegate porphyria is characterized by photosensitive cutaneous manifestations.

Although Gunther<sup>7</sup> and Waldenström<sup>71</sup> recognized cases of porphyria in which neurologic and cutaneous manifestations oc-



**Fig 32-3** Cutaneous manifestations of variegate porphyria. **A**, Bulla on index finger, pigmented scars, and collapsed blisters at finger tips in a 27 year old woman. **B**, Hands of a 36 year old man, note the erosions on backs of hands, the depigmented scars of past lesions, and the subungual involvement (Photographs kindly supplied by Lennox Eales, University of Cape Town, South Africa)



curred together, it was the extensive investigations of Dean and Barnes in South Africa that provided the most complete information about this disease and clearly distinguished it from acute intermittent porphyria.<sup>81</sup> Indirect evidence suggests that George III and other members of the royal houses of Stuart, Hanover, and Prussia may have suffered from this form of porphyria.<sup>93</sup>

### Genetics and Prevalence

Variegate porphyria is inherited as an autosomal dominant trait. The studies in South Africa identified at least 236 patients in 13 families, all of whom trace their lineage to a single couple who migrated from Holland in 1688.<sup>81</sup> Among the white (Afrikaner) population of South Africa, there are an estimated 12,000 persons with variegate porphyria in a population of 3,000,000, a prevalence of 1:250. The illness is also seen in the "Cape Coloured" race in Capetown, South Africa, but not in the Bantu.<sup>86</sup> It is considerably less common outside of South Africa; however, families with a similar or identical illness have been reported from Sweden,<sup>81</sup> Holland,<sup>87</sup> Great Britain,<sup>83</sup> Denmark,<sup>93</sup> Taiwan,<sup>88</sup> and the United States.<sup>22,82</sup> The symptoms first appear in late puberty or early adult life.

### Clinical Description

In general, cutaneous manifestations tend to predominate in males whereas acute neurologic episodes are more frequent in females. This difference may result from the relatively greater use of drugs by women and a greater exposure to sun by men, rather than from an inherent difference in the disease in the two sexes.

Skin lesions<sup>16,86</sup> are the only manifestations of the disease in about half of the affected individuals. The lesions are limited to exposed areas, especially the face and hands (Fig. 32-3). Trivial mechanical trauma to these areas results in the formation of bullae, 2 to 30 mm in diameter. The bullous fluid usually is serous or blood-tinged and not

particularly rich in porphyrins.<sup>16</sup> The bullous lesions tend to rupture, often become secondarily infected, and form shallow ulcers which heal slowly and leave scars. In addition to these lesions, exposure to the sun may be followed by an acute reaction manifested by erythema and edema. Facial hirsutism and hyperpigmentation of exposed areas are common findings.

The clinical manifestations of the acute attack of variegate porphyria do not differ in any important respect from those of acute intermittent porphyria (page 1025). However, such attacks are probably somewhat less common in variegate porphyria than in intermittent porphyria and almost always occur after exposure to a known porphyrogenic drug (Table 32-3).<sup>87</sup> Mortality during an attack approaches 25%.<sup>87</sup>

### Laboratory Findings

The most characteristic and consistent abnormality in porphyrin metabolism is an increase in fecal coproporphyrin and protoporphyrin. Fecal coproporphyrin ranges from 70 to over 1000  $\mu\text{g/g}$  dry weight (normal < 100  $\mu\text{g/g}$ ).<sup>84,140</sup> Fecal uroporphyrins may also be increased.<sup>96</sup> In addition, these patients excrete excessive amounts of a hydrophilic, dicarboxylic porphyrin conjugated with a peptide ("X-porphyrin").<sup>93</sup> These abnormalities are present even when the patient has no symptoms.

During acute attacks, ALA and PBG appear in the urine. However, in contrast to acute intermittent porphyria, urinary ALA and PBG levels frequently return to normal a few weeks after the onset of an attack, even though neurologic manifestations continue.<sup>88</sup> Furthermore, urine ALA and PBG levels are characteristically normal or only slightly increased in patients whose only manifestations are cutaneous.<sup>84,86</sup>

### Nature of the Metabolic Abnormality

As in acute intermittent porphyria, ALA synthetase activity is increased in the liver (Fig. 32-1, reaction 1).<sup>85,94</sup> This may be the

only abnormality, since the pattern of porphyrin excretion is similar to that in normal subjects given ALA.<sup>37</sup> (See discussion, page 1028.)

### Treatment

Acute attacks should be prevented and treated as outlined for acute intermittent porphyria. Since the "glucose effect" (page 1029) has been shown to operate in variegate porphyria,<sup>91</sup> a high-carbohydrate, high-protein diet is also advised for these patients.

In addition, subjects with cutaneous manifestations should be instructed to avoid sun exposure. The measures and topical agents described in connection with congenital erythropoietic porphyria (page 1025) may be useful for these patients.

## Hereditary Coproporphyria

This form of porphyria is characterized by the continuous, excessive fecal excretion of coproporphyrin III. Protoporphyrin excretion remains normal or only slightly increased.

The first comprehensive description of the disorder was that of Berger and Goldberg.<sup>81</sup> Since then, more than 40 cases have been reported.<sup>89,90</sup> The disease resembles acute intermittent porphyria and variegate porphyria in several important respects: (1) it is inherited as an autosomal dominant trait; (2) the excessive porphyrins are formed in the liver, probably because of excessive ALA synthetase activity<sup>92</sup>; (3) acute attacks occur which may be precipitated by barbiturates, anticonvulsants, sedatives (Table 32-3), and, unlike acute intermittent and variegate porphyria, chlorpromazine.<sup>89</sup> During these attacks ALA and PBG are excreted into the urine.

Over 60% of the patients with hereditary coproporphyria have no symptoms.<sup>89,90</sup> Most of the remainder complain of intermittent attacks, often beginning in childhood, that are similar to those seen in patients with acute intermittent porphyria. Photosensitivity has been observed but is less common than in

variegated porphyria. Fecal coproporphyrin is increased to between 100 and 3000  $\mu\text{g/g}$  dry weight (normal  $<40 \mu\text{g/g}$ ), but fecal protoporphyrin is normal or only slightly increased. Urine coproporphyrin may also be increased during symptomatic periods but usually is normal during remissions.

The primary genetic lesion is not completely understood. It has been suggested that there may be a partial block in the conversion of coproporphyrin to protoporphyrin<sup>17</sup> (Fig. 32-1, reaction 6), but this reaction has not been measured directly. Another hypothesis holds that the illness results from a mitochondrial defect that impairs uptake of coproporphyrinogen for subsequent incorporation into heme.<sup>90</sup>

Hereditary coproporphyria should be managed as outlined under acute intermittent porphyria.

## Protoporphyria

This inherited illness is characterized by greatly increased levels of free protoporphyrin in erythrocytes and feces. It was first described in 1961 by Magnus and coworkers.<sup>114</sup>

### Prevalence

Although accurate incidence figures are not available, protoporphyria appears to be relatively common. In the five-year period following the initial description, 65 cases were reported.<sup>103</sup> It appears to be inherited as an autosomal dominant trait<sup>107,122</sup>; however, many of the carriers have no clinical manifestations. In a study of nine families, there were 16 clinically affected individuals and 43 asymptomatic carriers.<sup>122</sup> For reasons which are not clear, protoporphyria is about twice as common in males as in females.<sup>103,104,122</sup> The disease usually is first manifested in childhood or adolescence.<sup>16,17</sup>

### Clinical Description

Patients with protoporphyria complain of a variety of cutaneous manifestations of

photosensitivity. In general, these are quite unlike those seen in patients with other porphyrias; bullae, scarring, sensitivity to trauma, hirsutism, and hyperpigmentation are uncommon. In some patients, the symptoms are subjective only.<sup>106</sup> After exposure to the sun, even through window glass,<sup>112,119</sup> for periods of a few minutes up to several hours, the patient notes intensely unpleasant sensations (photoparesthesias) such as prickling, itching, or burning. These sensations may persist for one to 24 hours, rarely as long as a week, and are partially relieved by cooling the skin.<sup>16</sup> In most patients, objective changes occur as well. Erythema resembling ordinary sunburn often appears during or shortly after exposure. Diffuse edema may develop two to three hours later. Less commonly, solar urticaria, a confluent, hive-like rash, occurs within minutes of exposure and lasts about one-half hour.<sup>114</sup> In an occasional patient, these early reactions are followed within a day or two by vesicular, eczematous lesions (solar eczema) which become crusted and last for several weeks.<sup>121</sup> Fine facial scarring and an appearance of premature aging may be seen in adults.<sup>122</sup> The cutaneous manifestations of protoporphyria do not differ greatly from those found in patients with photosensitivity of unknown cause (so-called "polymorphic light eruptions"), which is much more common and is not associated with abnormal porphyrin metabolism.<sup>119</sup>

Acute attacks of neurologic and abdominal symptoms do not occur in protoporphyric patients. The only non-cutaneous manifestations affect the hepatobiliary system. There is a tendency to develop cholelithiasis early in life,<sup>104,105,112</sup> the gallstones perhaps forming around a core of precipitated protoporphyrin. Fatal liver failure with a hepatitis-like picture or micronodular cirrhosis has been observed in several patients.<sup>101,108,125</sup> In these individuals, massive hepatic deposits of protoporphyrin were found.

### Laboratory Findings

In most of the reported patients with protoporphyria, there have been no quantitative or

morphologic abnormalities of the blood. In a patient with atypical findings, hemolytic anemia was detected and was relieved by splenectomy.<sup>118</sup>

The free erythrocyte protoporphyrin (FEP) level is greatly increased in symptomatic patients. Reported values range from 300 to 4500  $\mu\text{g}/\text{dl}$  (normal, less than 50  $\mu\text{g}/\text{dl}$ ).<sup>117,112,122</sup> Increased values for FEP may be observed in certain other conditions (Chapter 16), especially iron-deficiency anemia, but only rarely do they exceed 300  $\mu\text{g}/\text{dl}$ . In lead poisoning, however, FEP may be as high as in protoporphyria.<sup>117</sup> The FEP in asymptomatic carriers of protoporphyria ranges from normal levels to 200  $\mu\text{g}/\text{dl}$ .<sup>117,112,122</sup> In protoporphyria, the excessive erythrocyte protoporphyrin is not found in all the red cells. When examined with the fluorescence microscope, 7 to 60% of all the erythrocytes fluoresce brilliantly with the characteristic salmon-pink color of porphyrins. The remaining, non-fluorescent cells probably contain nearly normal concentrations of protoporphyrin.<sup>111</sup> In asymptomatic carriers, a small population of "fluorescytes" may be detected even when the total FEP level is normal.<sup>122,126</sup> The high concentration of porphyrin in individual erythrocytes renders them sensitive to hemolysis when exposed *in vitro* to 400 nm wavelength irradiation (photohemolysis).<sup>110,111</sup> This phenomenon probably results from lipid peroxidation at the red cell membrane.

Fecal protoporphyrin levels usually, but not always, are increased in symptomatic patients. Values usually range from 30 to 300  $\mu\text{g}/\text{g}$  (dry weight),<sup>112</sup> but may occasionally be as high as 1400  $\mu\text{g}/\text{g}$  (normal, less than 100  $\mu\text{g}/\text{g}$ ).<sup>104,107</sup> In some carriers, fecal porphyrins are elevated even when erythrocyte porphyrins are normal.<sup>107</sup> The fecal porphyrin excretion fluctuates considerably from one time period to another in the same patient,<sup>104</sup> and may be related to protein and carbohydrate intake ("glucose effect"). Following a period of fasting, an abrupt, pronounced increase in fecal protoporphyrin was observed in a study of a single patient.<sup>120</sup> Plasma protoporphyrin levels also may be increased.<sup>117</sup>



## Pathogenesis

Because of the original demonstration of protoporphyrin in erythrocytes, it was initially believed that the pigment is formed only in the erythropoietic system.<sup>114</sup> Kinetic and morphologic studies have suggested to some investigators that other organs, especially the liver, may also produce excess amounts of protoporphyrin,<sup>104,109,123</sup> but similar studies by others were considered to be consistent with the view that erythropoietic tissue is the sole source.<sup>124</sup>

The nature of the primary genetic defect in heme synthesis remains unknown. It is tempting to implicate a defect in the conversion of protoporphyrin to heme (the heme synthetase or ferrochelatase reaction, Fig. 32-1, reaction 7). Such a defect was looked for and not found in one patient,<sup>118</sup> but indirect evidence for a relative deficiency in this reaction was reported in another study.<sup>121</sup> ALA synthetase activity (Fig. 32-1, reaction 1) has been found to be increased in the plasma,<sup>118</sup> liver,<sup>104,115</sup> and blood.<sup>121</sup> The observed "glucose effect" also suggests that increased ALA synthetase activity plays a role in the pathogenesis of the disease.<sup>120</sup> However, as in patients with acute intermittent porphyria (page 1028), increased ALA synthetase activity may be a secondary rather than the primary abnormality.

## Treatment

Induction of carotenemia is effective in reducing the photosensitivity in protoporphyrin.<sup>113</sup> This may be accomplished by administration of  $\beta$ -carotene beads,<sup>113</sup> 15 to 180 mg/day, or of foods rich in carotenes, such as carrot juice.<sup>102</sup> In 29 of 30 patients so treated, sun tolerance was prolonged from less than 30 minutes to five to eight hours or more.

Since the "glucose effect" has been demonstrated in persons having erythropoietic porphyria,<sup>120</sup> it is possible that long-term administration of high-protein, high-carbohydrate diets might bring about reduction in FEP levels, but experience with such a regimen has not yet been reported.

## Acquired Porphyria

(Porphyria Cutanea Tarda; Porphyria Cutanea Tarda Symptomatica; Symptomatic Porphyria; Idiosyncratic, Idiopathic, or Constitutional Porphyria)

Acquired porphyria is characterized clinically by photosensitive skin lesions and biochemically by the excessive hepatic synthesis and urinary excretion of porphyrins, especially uroporphyrin I. This form of porphyria always occurs in association with liver disease, usually of alcoholic or toxic origin.

## Etiologic Factors and Prevalence

Acquired porphyria probably is the most commonly recognized disorder of porphyrin metabolism.<sup>136</sup> It is particularly common among the Bantu population of South Africa,<sup>86</sup> where it appears to be associated with hepatic iron overload and the excessive consumption of a variety of home-brewed alcoholic beverages.<sup>86,133</sup> It also occurs in other countries in patients with alcoholic liver disease<sup>17,41,133</sup>; however, it is an uncommon complication of this disorder. In one study of 360 patients with alcoholic cirrhosis, porphyria was detected in only seven.<sup>171</sup> Possibly reflecting a difference in alcohol consumption, acquired porphyria is considerably more common in males than in females. The disease tends to begin in middle life, especially in persons between 40 and 50 years of age.

The observation that only a small proportion of alcoholic patients acquire porphyria has led a number of investigators to suggest that an inherited, constitutional idiosyncrasy may be an etiologic factor.<sup>22,171,173</sup> As yet, there is little direct evidence of such an abnormality. Most family studies have not demonstrated evidence for such an idiosyncrasy. However, acquired porphyria has been described in one set of identical twins, both alcoholics,<sup>171</sup> and a sibling of another patient had slightly increased values for urinary uroporphyrin and fecal coproporphyrin.<sup>22</sup> In the African patients, no evidence of a familial incidence has been found, even when alcohol

consumption among family members was similar.<sup>86,153</sup>

In Turkey, between 1956 and 1961, an epidemic of acquired porphyria involving more than 3000 persons occurred as the result of exposure to a seed wheat fungicide, hexachlorobenzene.<sup>134,135,159,163</sup> Members of both sexes were affected, and many of the subjects were children. The syndrome was later reproduced in rats by administering hexachlorobenzene.<sup>157,162</sup>

Estrogens have been implicated in 29 patients with acquired porphyria.<sup>160,165</sup> Of these, 23 were men, 20 of whom had been treated with estrogen preparations, usually diethylstilbestrol, for carcinoma of the prostate.<sup>160</sup> However, porphyria has also been described in women receiving estrogens, including natural estrogens, for symptoms of the menopause.<sup>163</sup>

Acquired porphyria also has been associated with exposure to several other chemical agents and with certain illnesses (Table 32-4). These occurrences are relatively uncommon, having been reported only in one or a few patients.

### Clinical Description

The manifestations of the disturbance in porphyrin metabolism are limited to the skin and urine. The observed photosensitive skin lesions are indistinguishable from those associated with variegate porphyria (page 1031). Almost all the patients notice that their urine has a distinct red color.<sup>153</sup> These manifestations are accompanied by signs and symptoms of the underlying liver disease. There is no good correlation, however, between the occurrence of porphyria and the degree of liver disease.

### Laboratory Findings

These patients excrete greatly increased amounts of porphyrins, especially uroporphyrin, in the urine. In a study of 66 patients, the urine uroporphyrin averaged 2819  $\mu\text{g}/\text{l}$ , and exceeded 1000  $\mu\text{g}/\text{l}$  in 70% of the group (normal <40  $\mu\text{g}/24$  hr).<sup>139</sup> Urine copro-

**Table 32-4. Etiologic Factors in Acquired Porphyria**

<b>A Diseases</b>	
Alcoholic cirrhosis <sup>44</sup>	86, 133
Lupus erythematosus <sup>142,149</sup>	
Hepatic adenoma <sup>158</sup>	
Refractory anemia <sup>144,152</sup>	
Hemolytic anemia <sup>132,135a,147</sup>	
Chronic myelocytic leukemia <sup>151</sup>	
<b>B Drugs</b>	
Estrogens <sup>160</sup>	165
Busulfan <sup>151</sup>	
Sulfonal <sup>146</sup>	
Phenobarbitone <sup>146</sup>	
Tolbutamide <sup>146</sup>	
Chlorpropamide <sup>146</sup>	
Difantin <sup>146</sup>	
<b>C Toxins</b>	
Hexachlorobenzene <sup>135,159</sup>	163
Polychlorinated phenols <sup>131,154</sup>	

porphyrin averaged 560  $\mu\text{g}/\text{l}$  (normal <200  $\mu\text{g}/24$  hr). With more precise chemical techniques, uroporphyrin I was found to be the predominant porphyrin excreted.<sup>138,156</sup> However, increased amounts of uroporphyrin III, coproporphyrins I and III, and porphyrins with 7, 6, and 5 carboxyl groups also were found. In urine from rats with hexachlorobenzene-induced porphyria, type III isomers predominated.<sup>162</sup>

Normal or only slightly increased amounts of porphyrins are excreted in the feces.<sup>140</sup> However, unusual tetracarboxylic porphyrins, distinct from coproporphyrin, have been isolated from the feces of patients with acquired porphyria and from rats given hexachlorobenzene.<sup>141</sup>

The serum iron concentration often is increased. It exceeded 300  $\mu\text{g}/\text{dl}$  in 25% of Africans with the disease.<sup>153</sup> In the United States, in four patients from a series of 20, transferrin saturation was greater than 70%.<sup>143</sup>

Laboratory findings indicating liver disease vary considerably from one patient to another. There may be mild degrees of jaundice and slight to moderate elevations in serum transaminase levels. Liver biopsy usually demonstrates portal fibrosis, hepatic cell

necrosis, and varying degrees of iron overload.<sup>153,169</sup>

### Pathogenesis

Iron overload has been implicated in the pathogenesis of porphyria associated with alcoholic liver disease on the basis of several observations (1) excessive hepatic iron is almost invariably found<sup>142,169</sup>; (2) removal of iron by phlebotomy<sup>113,172</sup> or the administration of chelating agents<sup>167</sup> induces clinical and biochemical remission; and (3) administration of iron is followed by relapse.<sup>146</sup> Furthermore, in an *in vitro* system derived from hepatic tissue, ferrous iron was shown to inhibit the enzymes, uroporphyrinogen cosynthetase and decarboxylase (Fig. 32-1, reactions 4 and 5), and to increase the total synthesis of porphyrin from PBG.<sup>150</sup>

It seems reasonable that this effect of iron accounts for some of the observed abnormalities in patients with acquired porphyria. However, since iron overload by itself does not induce porphyria, the cellular and subcellular location of the iron as well as its chemical form may be critical to the development of the disease.

The activity of ALA synthetase has been studied by several investigators with conflicting results.<sup>68,92,137,165</sup>

### Treatment

Exposure to a toxic agent, if identified, should be eliminated. Abstinence from alcohol can lead to remissions in patients with porphyria associated with alcoholic liver disease<sup>171</sup>; however, removal of iron by phlebotomy can induce remissions even if alcohol intake continues. In general, 500 ml of blood can be removed every two weeks. In one series of 20 patients, remission was achieved in 18 by the removal of 2.5 to 8.5 l of blood over a period of three to eight and one-half months.<sup>143</sup> Of these, 14 remained in remission over periods of one to three and one-quarter years; of two in whom relapse occurred, one was successfully retreated.

Chloroquine has been employed in the treatment of patients with acquired porphyria,

but its use entails significant risks. Following the administration of 0.5 to 1.0 g/day for several days, a large portion of the uroporphyrin stored in the liver is excreted in the urine.<sup>145,168,170</sup> This "purging" effect apparently results from the formation of an easily excreted, water-soluble complex between uroporphyrin and the drug.<sup>164</sup> Following the chloroquine purge, a clinical and biochemical remission lasting up to one year may occur. Unfortunately, the chloroquine effect is accompanied by malaise, anorexia, fever, and signs of hepatocellular damage,<sup>145,168</sup> possibly because of injury to hepatic mitochondria.<sup>164</sup> These reactions are usually transient and the patients recover within a few days. Hemolytic anemia and ascites also have been reported.<sup>170</sup> Because of the potential danger of the hepatic reaction and also because phlebotomy appears to be relatively safe and effective, chloroquine must be considered to be an experimental and somewhat dangerous form of therapy. It should be used only under highly selected and carefully controlled circumstances. It is possible that the use of lower doses may make the drug safer without impairing its efficacy.<sup>161</sup>

Still another form of therapy is alkalization of the urine by administration of sodium bicarbonate, usually in a dose of 1.3 g three times daily.<sup>158</sup> With this treatment, eight of 10 patients improved clinically and biochemically. However, it was considered possible that the observed responses were related to changes in alcohol consumption, and the need for a controlled study was acknowledged.

In another uncontrolled study, eight patients with hexachlorobenzene-induced porphyria improved when treated with the chelating agent, disodium ethylenediamine tetraacetic acid (EDTA).<sup>159</sup>

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# Part IV

## Disorders of Platelets and Hemostasis

Except for that which occurs during menstruation, spontaneous bleeding is abnormal. Surprisingly little blood is lost from even large injuries. This is a consequence of the efficiency with which vascular integrity is normally maintained and the rapidity with which it is restored following injury. In general, these phenomena reflect the functional effectiveness of the hemostatic apparatus (Chapters 9 and 10). It must be recognized, however, that the adequacy of hemostasis is only relative, and, despite the presence of normal vessels, platelets, and coagulation factors, bleeding can occur as the result of localized pathologic processes.

The following seven chapters will deal with disorders which result from abnormalities of the hemostatic process. Chapter 33 will summarize the diagnostic approach to these disorders, and includes a brief discussion of laboratory methods for their study. In the chapters which follow, individual disorders will be considered in five categories: quantitative variations of platelets in disease (Chapter 34), qualitative disorders of platelet function (Chapter 35), bleeding disorders due to vascular abnormalities (Chapter 36), hereditary coagulation disorders (Chapter 37), and the acquired coagulation disorders (Chapter 38). The pathophysiology of thrombosis and the principles of antithrombotic therapy, insofar as they pertain to the hemostatic apparatus, are summarized in Chapter 39.

## SECTION 1: *Approach to Problems of Hemostasis and Coagulation*



# *The Diagnostic Approach to the Bleeding Disorders*

Clinical Evaluation of the Bleeding Patient  
Manifestations of Disordered Hemostasis  
Clinical Features of the Hereditary Bleeding Disorders  
Clinical Features of the Acquired Bleeding Disorders  
Laboratory Methods for the Study of Hemostasis and Blood Coagulation  
Tests of the Vascular and Platelet Phases  
Tests of the Coagulation Phase  
Initial Laboratory Approach  
The Utility of Screening Tests  
Confirmatory Tests  
Laboratory Evaluation in the Newborn

### **Clinical Evaluation of the Bleeding Patient**

The importance of clinical evaluation in patients with bleeding disorders should neither be overemphasized nor minimized. The presence of a generalized disorder of hemostasis is often immediately and dramatically obvious, and in many cases there is nothing about the clinical picture to suggest any particular type of bleeding disorder. Nevertheless, a careful evaluation of the presenting complaint can often provide valuable clues as to whether the abnormality resides in the vessels, platelets, or the process of blood coagulation; a carefully obtained history can usually establish whether the disorder is hereditary or acquired; the physical examina-

tion may reveal findings such as the characteristic skin lesions of hereditary hemorrhagic telangiectasia, which alone may provide the diagnosis in a previously perplexing bleeding problem. The laboratory should supplement and not supersede a careful review of the history and the physical examination, if it is to be used to maximum advantage in terms of time and expense.

### **Manifestations of Disordered Hemostasis**

Certain signs and symptoms are virtually diagnostic of disordered hemostasis. They can be arbitrarily divided into two groups: those which are more frequently seen in disorders of blood coagulation, and those which are commonest in disorders of the vessels and platelets. The latter group is frequently referred to by the descriptive term "purpuric disorders," owing to the prominence of cutaneous and mucosal bleeding. The clinical findings that are most valuable in distinguishing between these two broad categories are summarized in Table 33-1. Although these criteria are relative, they provide valuable clues to the probable diagnosis<sup>5,8,14</sup> if applied to the predominating clinical features in a given patient.

### **Bleeding into Skin and Soft Tissues**

✎ **Petechiae** are characteristic of an abnormality in the vessels or the platelets, eg, throm-



**Table 33-1. The Clinical Distinction between Disorders of Vessels and Platelets and Disorders of Blood Coagulation**

<i>Findings</i>	<i>Disorders of Coagulation</i>	<i>Disorders of Platelets or Vessels ('Purpura' disorders)</i>
Petechiae	Rare	Characteristic
Deep dissecting hematomas	Characteristic	Rare
Superficial ecchymoses	Common, usually large and solitary	Characteristic, usually small and multiple
Hemarthrosis	Characteristic	Rare
Delayed bleeding	Common	Rare
Bleeding from superficial cuts and scratches	Minimal	Persistent, often profuse
Sex of patient	80-90% of hereditary forms occur only in males	Relatively more common in females
Positive family history	Common	Rare

bocytopenia, and are exceedingly rare in the coagulation disorders. These lesions (Fig. 33-1) are small capillary hemorrhages which range from the size of a pinhead to much larger and characteristically develop and regress in crops. They are most conspicuous in areas of increased venous pressure, eg, the dependent portions of the body and areas subjected to pressure or constriction from girdles or stockings. In scurvy, petechiae may be distributed around hair follicles in the "saddle area" of the thighs and buttocks (Fig. 36-10, page 1150). Petechiae must be distinguished from small telangiectases and angiomatous (Table 36-3, page 1147).

In the purpuric disorders, petechiae commonly are associated with multiple superficial ecchymoses, which usually develop without perceptible trauma but seldom spread into deeper tissues. Small isolated ecchymoses are common in apparently normal women, especially on the legs, and in small children.

Although large superficial ecchymoses may be seen in the coagulation disorders, the most characteristic lesion is the large spreading hematoma (Fig. 33-2). Such hematomas may arise spontaneously or follow very trivial trauma and frequently spread to involve an entire limb by dissecting muscles and deep fascial spaces, often with little discoloration of the overlying skin.

### Hemarthrosis

Hemorrhage into synovial joints is virtually diagnostic of a severe hereditary coagulation disorder, most commonly hemophilia A or hemophilia B, and is rare in disorders of the vessels and platelets or in acquired coagulation disorders. This disabling symptom (Fig. 33-3) often develops without discoloration or other external evidence of bleeding, and the patient may attribute the symptoms to arthritis rather than to bleeding. Subperiosteal hemorrhages in children with scurvy, and swollen painful joints which may develop in some patients with allergic purpura, occasionally may be confused with hemarthrosis (Chapter 36).

### Traumatic Bleeding

The unavoidable trauma of daily life and even minor surgical procedures are a greater challenge to hemostasis than any test yet contrived in the laboratory. In contrast to "spontaneous" bleeding manifestations, bleeding following trauma in a person with a hemorrhagic diathesis differs in a quantitative way from that which would normally be expected, ie, in terms of amount, duration, and magnitude of the inciting trauma. It is extremely difficult to assess such variables

accurately, however. The amount of blood lost usually is exaggerated by the patient. Whether or not transfusions were required and the number administered may serve as a rough guide. The patient's statement concerning the duration of bleeding is more reliable. Detailed inquiry as to past injuries and

operations must be made, since the patient is likely to forget procedures or injuries which were uncomplicated and dwell on those in which bleeding was a problem.

In the coagulation disorders, the onset of bleeding following trauma frequently is delayed. For example, bleeding following a



Fig 33-1. Strongly positive reaction to the tourniquet test in a patient with chronic idiopathic thrombocytopenic purpura. The platelet count was  $40 \times 10^9/l$ , the bleeding time 42 minutes. Note that there are few petechiae in the area compressed by the blood pressure cuff.

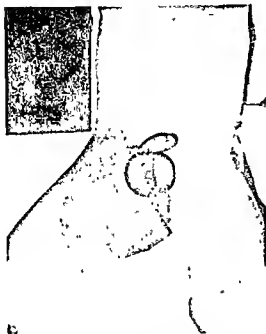


Fig 33-2 Large dissecting hematoma of thigh in a patient with hemophilia A. The lesion resulted from a slight bump to the inguinal area, and spread to involve the entire thigh (Courtesy of Dr John Lukens)

tooth extraction may stop completely only to recur in a matter of hours and persist despite the use of styptics, vasoconstrictors, and packing. The temporary hemostatic adequacy of the platelet thrombus despite defective blood coagulation may explain this phenomenon of delayed bleeding, as well as the fact that patients with coagulation disorders seldom bleed abnormally from small superficial cuts, eg, razor nicks. In contrast, post-traumatic or post-surgical bleeding in thrombocytopenia usually is immediate in onset, as a rule responds to local measures and rarely is as rapid or voluminous as that encountered in the coagulation disorders, but it may persist for hours or days from surprisingly small injuries.

Valuable information often is obtained by a careful review of dental procedures, since most patients will have had one or more teeth extracted at some time during their life. Inquiry should clarify whether the extractions were single or multiple, the size and location of the tooth or teeth, any therapy given, and

the amount, if any, of direct operative trauma.<sup>121</sup> The amount of bleeding normally encountered varies greatly, but as a rough guide it may be stated that the uncomplicated extraction of a single molar tooth may result in brisk bleeding for up to one hour and slight oozing for up to two days in normal persons.<sup>118</sup> Bleeding normally is more profuse from upper than from lower sockets and is more marked following extraction of molar teeth, particularly impacted third molars ("wisdom teeth"), than after removal of other teeth. In the hereditary coagulation disorders, the shedding of deciduous teeth is frequently uncomplicated.

The response to trauma is an excellent "screening test" for the presence of a hereditary hemorrhagic disorder, and a history of surgical procedures or significant injury without abnormal bleeding is equally good evidence against the presence of such a disorder. The removal of molar teeth is a "major" challenge to hemostasis, as is a tonsillectomy, and it is a rare hemophiliac, however mildly affected, who can withstand these procedures without undue bleeding.

### Miscellaneous Bleeding Manifestations

Spontaneous bleeding from bodily orifices may complicate any significant hemorrhagic diathesis, eg, menorrhagia, metrorrhagia, hematuria, hematemesis, melena, epistaxis, gingival bleeding. These symptoms occur with approximately equal frequency in purpuric disorders and coagulation disorders, and when they are the first or predominant manifestations they may erroneously be attributed to a more familiar local lesion. Severe menorrhagia may be the sole complaint of women with von Willebrand's disease, mild thrombocytopenia, or autosomally inherited coagulation disorders. Recurrent gastrointestinal bleeding or epistaxis in the absence of other bleeding manifestations is common in hereditary hemorrhagic telangiectasia. A coagulation disorder or a disorder of platelet function should be looked for if protracted hematuria is the only symptom.

Bleeding into serous cavities and internal

fascial spaces may create serious diagnostic problems, and is not uncommon in the hereditary coagulation disorders. In hemophilia, retroperitoneal hemorrhage or bleeding into the psoas sheath may mimic appendicitis, and hemorrhage into the bowel wall may be confused with intestinal obstruction. Signs and symptoms which simulate a variety of acute intra-abdominal disorders may also be seen in allergic purpura (Chapter 36). Bleeding into the central nervous system may complicate thrombocytopenia, and may follow minor trauma in the coagulation disorders. Multiple small retinal hemorrhages are common in thrombocytopenia and other purpuric disorders, but are uncommon in the hereditary coagulation disorders. Large hematomas of the orbit may be seen in the latter. The coexistence of bleeding and thromboembolic phenomena, or bleeding from previously intact venipuncture sites, suggests the presence of diffuse intravascular coagulation (DIC).<sup>44,102</sup> Protracted wound healing and

abnormal scar formation have been described in hereditary afibrinogenemia, the dysfibrinogenemias, and in factor XIII deficiency<sup>48</sup> (Chapter 37). Hemoptysis is rarely due to a hemorrhagic disorder.

### Clinical Features of the Hereditary Bleeding Disorders

A hereditary bleeding disorder is suggested by the onset of bleeding symptoms in infancy and childhood, a positive family history, particularly if it reveals a consistent genetic pattern, and laboratory evidence of a single or "isolated" abnormality, most commonly the deficiency of a single coagulation factor.

#### Age at Onset: Bleeding in the Neonate

Birth and the neonatal period provide unique challenges to the hemostatic mecha-

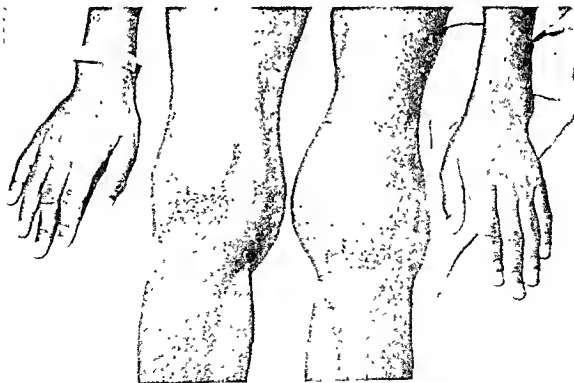


Fig 33-3. Acute hemarthrosis and its sequelae in a patient with hemophilia B. Note the periarthral swelling in the left leg and the marked atrophy of the thigh muscles as a result of recurrent hemarthrosis (Courtesy of Dr. John Lukens).

nism,<sup>68,101</sup> and bleeding during the first month of life often provides the first evidence of a hereditary disorder of hemostasis. Small cephalohematomas and petechiae are common in the newborn as a result of the trauma of delivery.<sup>68</sup> Large cephalohematomas which progressively increase in size may result from hemophilia, but are more common in acquired bleeding disorders such as hemorrhagic disease of the newborn (Chapter 38). Bleeding from the umbilical stump and following circumcision is characteristic of the latter syndrome, but is uncommon in the hereditary coagulation disorders,<sup>68</sup> with the exception of hypofibrinogenemia<sup>17</sup> and factor XIII deficiency.<sup>48</sup> In these disorders, the onset of cord bleeding may be delayed. In the evaluation of bleeding in the neonate, it should be remembered that hematochezia and hematemesis may originate from swallowed blood of maternal origin.

When a hereditary coagulation disorder is not associated with bleeding in the neonatal period, or when the significance of bleeding is overlooked, the disorder may become clinically "silent" for a time. Hematomas may be first seen only when the child becomes active. Hemarthrosis commonly does not develop until three to four years of age.

Rarely, the date of onset of bleeding may be difficult to establish, and it may be difficult to distinguish between a mild hereditary hemorrhagic disorder and the insidious onset of an acquired defect. Patients with mild hereditary coagulation disorders may enter adult life before characteristic bleeding manifestations occur. In these cases, and in some forms of hereditary thrombocytopenia and disordered platelet function, it is not uncommon to obtain a history of marked post-traumatic bruising and hematoma formation which have come to be accepted by the patient as "normal." In hereditary hemorrhagic telangiectasia, the lesions become more prominent with advancing age, and may not be symptomatic until middle age. Similarly, in the patient with the Ehlers-Danlos syndrome, bleeding may not be a problem until adult life.

## The Family History

This is of great importance in the evaluation of bleeding disorders. Details of the various genetic patterns which may be encountered are discussed elsewhere. In autosomal dominant traits with characteristic symptoms and high penetrance, such as hereditary hemorrhagic telangiectasia, an accurate pedigree spanning several generations can often be obtained (Fig. 36-5, page 1145). The presence of characteristic bleeding manifestations in male siblings and maternal uncles is virtually diagnostic of X-linked recessive inheritance which characterizes hemophilia A and hemophilia B. In such X-linked traits, the family history also may be helpful in a negative sense, ie, it may clearly exclude the disorder in certain offspring such as the sons of a known hemophiliac.

The limitations of the family history, however, are much greater than is commonly realized. "Hearsay" history is very difficult to evaluate, and it is difficult and often impossible to assess the significance of "easy bruising," or to differentiate between manifestations of a generalized bleeding disorder and more common localized lesions, eg, peptic ulcers, uterine leiomyomas. In affected families, a bewildering variety of unrelated symptoms are frequently attributed to bleeding.

It must be emphasized that a negative family history is of no value in excluding a hereditary coagulation disorder in an individual patient; eg, as many as 40% of patients with hemophilia A will have a negative family history.<sup>13</sup> The family history is usually negative in the autosomal recessive traits, and consanguinity, which is commonly present in these kindreds, is notoriously difficult to document or exclude.

## Clinical Features of Acquired Bleeding Disorders

Generalized bleeding may be a prominent feature of a wide variety of disorders which encompass virtually the entire field of med-

icine. Bleeding manifestations usually are less severe than in the hereditary forms, and the clinical picture often is dominated by evidence of the underlying disorder rather than by bleeding alone. In contrast to the hereditary forms, a multiple hemostatic defect is commonly present, often including thrombocytopenia as well as significant coagulation abnormalities.

In general, the study of the acquired bleeding disorders should emphasize the patient and not the laboratory. A carefully taken history and the physical examination will often reveal the cause of thrombocytopenia, eg, a drug, acute leukemia. In most vascular disorders, eg, senile purpura, allergic purpura, scurvy, and amyloidosis, the history and physical examination are of primary diagnostic importance whereas the laboratory has little to offer.

### Drug History

The importance of an exhaustive interrogation regarding drug use and chemical exposure cannot be overemphasized. The list of drugs which are associated with thrombocytopenia (Table 34-5, page 1084) or vascular purpura (Table 36-1, page 1137) grows longer each year. Less common but more serious is drug-induced aplastic anemia, which may first present with bleeding (Fig. 56-4, page 1755). A large number of commonly used drugs (Table 35-1, page 1120), notably aspirin, impair platelet function. These drugs are a heretofore unrecognized cause of mild purpura and also produce confusing laboratory abnormalities which may lead to expensive and unnecessary laboratory studies. The same drugs may provoke bleeding when administered to patients with hereditary coagulation disorders, eg, hemophilia A.

Coagulation abnormalities may also result from drug ingestion. Drugs which potentiate or antagonize the anticoagulant effects of coumarin anticoagulants may lead to bleeding or erratic laboratory control. The surreptitious ingestion of such anticoagulants is not uncommon (Chapter 39).

## Laboratory Methods for the Study of Hemostasis and Blood Coagulation

There is no single test which is suitable for the laboratory evaluation of the overall process of hemostasis and blood coagulation, but methods of varying complexity and utility are now available for assessing various components and functions individually. The following discussion will emphasize those methods which are simple and widely available in routine laboratories. The interpretation of the most commonly used tests and the range of values obtained in normal subjects with representative techniques are summarized in Table 33-2. Definitive methods usually require a specially equipped laboratory and trained personnel, and will be discussed here from a general standpoint only. Additional comments concerning the utility and limitations of the various methods will be found in later chapters dealing with individual disorders. For details concerning such definitive methods, the reader is referred to more comprehensive works devoted entirely to this subject.<sup>10,24,51,62,102,138</sup>

### Tests of the Vascular and Platelet Phases

#### The Bleeding Time

Hemostasis in a small superficial wound, such as that produced in measuring the bleeding time, depends on the rate at which a stable platelet thrombus is formed, and thus measures the efficiency of the vascular and platelet phases. It does not, however, discriminate between vascular defects, thrombocytopenia, and platelet dysfunction. The bleeding time leaves much to be desired in terms of reproducibility, since no two skin areas are exactly the same and it is impossible to produce a truly standard wound.<sup>135</sup>

Despite these intrinsic limitations, the bleeding time is valuable when carefully performed. When determined by the modified Ivy technique,<sup>37</sup> it is usually prolonged when the platelet count is below  $100 \times 10^9/l$ ,<sup>45</sup> as

Table 33-2. Interpretation of Common Tests of Hemostasis and Blood Coagulation

Test	Normal Range ( $\pm 2$ SD) and Method	Common Causes of Abnormalities*
1 Platelet count	140-440 $\times 10^9/l^{17,18}$	Thrombocytopenia Thrombocytosis
2 Bleeding time		
Ivy	1-9 mins <sup>19</sup> †	Thrombocytopenia, von Willebrand's disease, platelet dysfunction, vascular disorders (uncommon), severe coagulation disorders (rare)
Duke	1-4 mins <sup>15</sup>	
3 Partial thromboplastin time		
Standard	68-82 secs <sup>9¶</sup>	Deficiencies or inhibitors of factors XII, XI, IX, VIII, X, V, prothrom- bin, or fibrinogen
Activated	32-46 secs <sup>10¶</sup>	
4 Plasma prothrombin time	11-15 secs <sup>11‡</sup>	Deficiencies or inhibitors of factors VII, X, V, prothrombin or fibrinogen
5 Coagulation time		
Glass tubes	8-18 mins <sup>12§</sup>	As $\pm 3$ above, but abnormal only in severe deficiencies
6 Thromboplastin generation test <sup>7,10</sup>	§	Deficiencies of inhibitors of factors XII, XI, IX, VIII, V or X
Plasma abnormal	§	Deficiency of factors V or VIII
Serum abnormal†	§	Deficiency of factors X or IX
Plasma and serum abnormal†	§	Deficiency of factors XII, XI, or inhibitor
7 Plasma thrombin time	13-17 secs <sup>4‡</sup>	Afibrinogenemia, dysfibrinogenemia and hypofibrinogenemia, inhibi- tors of thrombin or fibrin poly- merization
8 Fibrinogen assay	160-415 mg/dl <sup>5‡,11§</sup>	Afibrinogenemia, dysfibrinogenemia and hypofibrinogenemia, inhibi- tors of thrombin or fibrin polymer- ization

\*Tests 3 through 8 are affected by heparin

†Deficiencies of factor XI or factor XII may produce either a combined serum and plasma abnormality or a serum abnormality alone

‡Confidence limits determined by logarithmic plot

§Normal range varies widely and must be interpreted in terms of control system

¶Minor variations depending on exact technique employed

‡Significant variations depending on thromboplastin used

well as in the majority of patients with von Willebrand's disease or disorders of platelet function. Contrary to theory, this test is only inconsistently abnormal in most disorders attributed to abnormalities of the vessels. The bleeding time is commonly prolonged in hereditary afibrinogenemia, probably as the result of platelet dysfunction (page 1127), and is occasionally prolonged in other severe coagulation disorders (Chapter 37).

### Related Methods

The template bleeding time,<sup>67,92</sup> the "secondary" bleeding time,<sup>19</sup> and various automated methods<sup>131</sup> are modifications of the Ivy method which may be more sensitive and reproducible than the original procedure. The carlobe bleeding time performed by the Duke method<sup>113</sup> is less sensitive than the modified Ivy method, particularly in the detection of

patients with von Willebrand's disease.<sup>97</sup> The aspirin tolerance test assesses the effect of a standard dose of aspirin on the Duke bleeding time.<sup>113</sup> This has been proposed as a method of detecting mild von Willebrand's disease, but the diagnostic value of the method is doubtful.<sup>92,143</sup> The administration of aspirin will prolong the bleeding time slightly in normal subjects and in patients with hemophilia A and hemophilia B,<sup>92</sup> as well as in patients with von Willebrand's disease, and may be dangerous in patients with coagulation disorders.

In the tourniquet test,<sup>127a</sup> a "standard" increase in venous and capillary pressure is produced for a short time by means of a sphygmomanometer.<sup>37</sup> In the presence of vascular abnormalities, or insufficient or abnormal platelets, petechiae may develop. The number and size of these lesions are an extremely crude index of the efficiency of the vascular and platelet phases. The tourniquet test correlates poorly with the platelet count; many normal subjects will develop some petechiae and many patients with bleeding due to vascular or platelet disorders will not. As a consequence, the tourniquet test is of little value as a screening test for disordered hemostasis.

Despite a promising beginning,<sup>82</sup> capillary microscopy has proved of little value in the study of bleeding disorders.

### Platelet Enumeration

Platelets are considerably more difficult to count than erythrocytes or leukocytes. This is to be expected in dealing with structures of such small size whose major physiologic attributes are adhesion to foreign surfaces and rapid aggregation following even minimal trauma.

In general, techniques for platelet counting may be divided into four groups, namely (1) *hemocytometer or direct methods*, in which whole blood is diluted and the platelets are counted in much the same way as leukocytes or erythrocytes; (2) *indirect methods*, in which red cells or white cells are enumerated directly and their proportion to platelets is

determined in a stained blood smear; (3) *semi-automated methods*, in which the number of platelets present in plasma prepared by sedimentation or centrifugation is determined in an electronic particle counter; and (4) *fully automated electronic methods*. Many other methods for platelet counting and innumerable technical modifications have been described, each with certain advantages and disadvantages.<sup>16,109</sup> An estimate of platelet numbers in a well-prepared blood smear by an experienced observer serves as a valuable check on the platelet count as determined by any method.

### Direct Methods

In the method of Brecher and Cronkite,<sup>27</sup> the difficulty in distinguishing platelets from other particles is minimized by the use of phase contrast microscopy and dilution of the blood in 1% ammonium oxalate, a reagent which hemolyzes the red cells and provides a clear background. A long working distance phase condenser, a 43 X medium dark contrast phase objective, flat-bottomed hemocytometers, and thin coverslips (No. 1 or 1½), rather than the usual hemocytometer coverglass, are required. Platelet counts can be carried out on either venous or capillary blood, but the latter is subject to the greater errors.<sup>27</sup> The earlobe should not be used as a source of capillary blood because the fine hair there is said to favor adhesion of the platelets. Venous blood may be collected without anticoagulant in a siliconized test tube<sup>27</sup> or on a siliconized slide, but the preferred method is the use of di- or tripotassium or disodium EDTA (2.5 to 5 mg/2 ml of whole blood). Double oxalate and citrate are not recommended as anticoagulants for platelet counting. The use of cocaine as a diluent may further improve the visualization of platelets.<sup>109</sup> The principles of phase contrast microscopy are described elsewhere<sup>37</sup> (Chapter 1).

Despite the special equipment required, the Brecher-Cronkite technique probably represents the best available compromise between cost, time, and accuracy, even though the



error of replication is still relatively high (1 CV = 7-17%).<sup>37,55,60,88</sup> In general, errors tend to result in low platelet counts. This may be because of a poorly collected blood sample, in which platelet aggregation has been induced by mechanical trauma or contaminating tissue "juice" or thrombin, or from adherence of platelets to particles of debris on the glassware used or in the diluting fluid. Erroneously high counts may result from fragmentation of platelets without complete dissolution, or the counting of particles of debris, eg, crystals of some salts making up the diluting fluid, dye, bacteria, portions of hemolyzed red cells, broken leukocytes. These errors can be minimized by the use of scrupulously clean glassware. Diluting fluids should always be refiltered before each determination.

The Rees-Ecker method is the progenitor of the above technique, which it resembles in most respects. Because the diluting fluid<sup>37</sup> is less satisfactory and an ordinary light microscope is used, there is much greater difficulty in visualizing the platelets. When counting platelets by this technique, the observer should control the fine adjustment of the microscope in order to obtain the critical focusing that reveals the characteristic highly refractile, silvery appearance of the platelets. The platelets are lilac-colored,  $\frac{1}{4}$  to  $\frac{1}{2}$  the diameter of the red corpuscles, and usually oval or rod- or comma-shaped. They may be seen singly or in groups. It is important to distinguish platelets from globules of oil, irregularly shaped debris floating on the upper layers of fluid, strings of cocci, and other particles which may be found in the fluid.

### Semi-automated Methods

In the method of Bull et al.,<sup>34</sup> whole blood is collected in EDTA and either allowed to settle spontaneously or is centrifuged briefly. The platelets in the supernatant platelet-rich plasma are then enumerated by means of an electronic particle counter.<sup>29,33,88</sup> The instrument must be equipped with a modified 50 to 70  $\mu$ m aperture tube, and should be

calibrated with an external standard such as latex particles, bacteria,<sup>83</sup> or formalinized platelets. Extreme caution and frequent blank runs are required to keep diluting solutions free of contaminating particles. Platelet cold agglutinins<sup>41</sup> and abnormal amounts of plasma proteins in the various paraproteinemias<sup>24</sup> may produce falsely low platelet counts, whereas carryover from sample to sample may result in overestimation of platelet numbers, particularly in thrombocytopenic samples.<sup>29</sup> Owing to the high dilution of the platelet-rich plasma required for electronic counting (1:3000 to 1:5000), the use of a highly accurate diluting device is recommended.<sup>26</sup> A slide rule which simplifies corrections for hematocrit and coincidence factors is now available commercially (Coulter).<sup>33</sup>

Semi-automated methods improve the precision of the platelet count substantially (CV of replicate samples = 1.2 to 4%<sup>33,55,60,83</sup>). However, they require more expensive equipment and represent little saving of time as compared with manual methods unless 30 or more counts must be made in one day.<sup>26</sup> Centrifuges must be calibrated carefully and frequently, since the centrifugal force employed in preparing the platelet-rich plasma is critical.<sup>26</sup> Significant errors may result from unpredictable variations in the percentage of platelets recovered in the supernatant plasma prepared by either sedimentation or centrifugation.<sup>50</sup>

### Fully Automated Methods

Instruments for totally automated platelet counting are now available commercially. For example, with the Hemalog (Technicon), whole blood samples are collected in EDTA, diluted, and hemolyzed in 2 M urea, and platelets are enumerated by means of an optical particle counter which employs the principle of reverse darkfield microscopy.<sup>31</sup> The precision of platelet counting with this instrument is equal to or even greater than that obtained with semi-automated methods.<sup>64,117</sup> Carryover of platelets from samples with high counts to subsequent samples has been

a problem, but can be eliminated by repeating the determination.

Although platelet counting is but one function of such "hematologic autoanalyzers," the disadvantages of these massive instruments are apparent, eg, size, great cost, considerable maintenance time. Their cost would seem justified only by a large volume of work, but their utility in this setting is undeniable. Evaluation of these instruments is still incomplete.

### Indirect Methods

These methods were originated prior to the advent of reliable direct methods, are quite inaccurate, and are not recommended. The simplest procedure is to note the number of platelets as compared with the number of red corpuscles or leukocytes in a stained blood smear. Fonio placed a drop of 14% aqueous solution of magnesium sulfate on the skin and punctured the finger through this in order to dilute the blood at once and to prevent aggregation of the platelets. Olef's method<sup>100</sup> is perhaps the best of the indirect procedures but is somewhat cumbersome. Dameshek's method<sup>40</sup> is similar but simpler.

Platelet counts made by indirect methods tend to be higher than those obtained by direct methods of enumeration. This is attributable in part to the tendency of red cells to concentrate at the edge of the smear, thus giving a falsely high ratio of platelets to red cells in the central areas where the count is likely to be made.<sup>26</sup>

### Normal Values for Platelets

Different normal values have been recorded, depending chiefly on the method used. Employing the Rees and Ecker method, the mean platelet count  $\pm 2$  SD in 80 healthy young adults was found to be  $241 \pm 100 \times 10^9/l$ ,<sup>125</sup> which would give a normal range of 140 to  $340 \times 10^9/l$ . Normal values for the Brecher-Cronkite method are  $250 \pm 90 \times 10^9/l$ , 95% of the counts ranging from 140 to  $440 \times 10^9/l$ .<sup>27</sup> Using his own method, Tocantins<sup>134</sup> found quite simi-

lar values. His figures were: cutaneous blood, 250; venous blood, 310; arterial blood, 350 with standard deviations of 58.5 to  $128 \times 10^9/l$ . Results obtained with the semi-automated method of Bull et al<sup>34</sup> were virtually identical to those obtained by Brecher and Cronkite. Similar normal values have been claimed for the totally automated instruments, but further documentation is needed.

Indirect counting methods have, in general, yielded much higher normal values. Thus, Olef,<sup>100</sup> using his own method, found an average count of  $514 \times 10^9/l$  (437 to 586). By Dameshek's method,<sup>40</sup> 500 to  $900 \times 10^9/l$  platelets were regarded as normal. As already stated, the highest counts recorded are not necessarily correct.

### Tests of Specific Platelet Functions

Owing to the rapid advances in knowledge concerning qualitative abnormalities of the platelets, the various techniques for measurement of specific platelet functions, formerly the province of the highly specialized research laboratory, are now being used with increasing frequency for diagnostic purposes.<sup>40a,149</sup>

### Platelet Adhesiveness

Numerous attempts have been made to measure the adhesion of platelets to foreign surfaces,<sup>69</sup> the greatest effort having been directed toward the development of a technique which detects the abnormality in primary hemostasis which is present in patients with von Willebrand's disease.<sup>18,118,143,149</sup> The diagnostic utility of these techniques in the various disorders of platelet function remains unclear.

The *in vivo* method of Borchgrevink<sup>18</sup> presumably measures the adhesion of platelets to the wound surface. Platelets are enumerated in the capillary blood issuing from a bleeding-time puncture, and adherent platelets are expressed as a percentage of the venous platelet count. As estimated by this

method, adhesion is influenced by all of the variables intrinsic to the bleeding time and by the hematocrit.<sup>69</sup> The results of the test are abnormal in many patients with von Willebrand's disease and in those with certain disorders of platelet function.

The retention of platelets within glass-bead columns (glass-bead "adhesion") has been studied by numerous methods, of which two appear to detect an abnormality in many patients with von Willebrand's disease. In the Salzman method,<sup>118</sup> venous blood is aspirated directly from the vein through a bead column and into a Vacutainer. Results are expressed as the percentage of the venous platelet count retained. The wide range of values obtained in normal subjects limits the usefulness of the technique. Modifications which provide a more standardized vacuum have been described.<sup>21</sup> Somewhat less cumbersome are *in vitro* methods in which heparinized whole blood<sup>21</sup> or "native" blood without anticoagulant<sup>70</sup> is passed through a bead column, and the percentage of retained platelets is determined. These methods are difficult to standardize,<sup>22,23</sup> and are exquisitely sensitive to *in vitro* platelet manipulation.<sup>131</sup> If measured in citrated plasma, retention appears to measure mainly the entrapment of platelet aggregates formed as the result of released ADP.<sup>63</sup>

The adhesion of platelets to collagen fibers *in vitro* is unaffected by strong chelating agents which completely inhibit ADP-induced aggregation. Thus, in platelet-rich plasma containing EDTA, adhesion to collagen may be assessed in the absence of aggregation. Techniques based on aggregometry<sup>127</sup> and the enumeration of free and adherent platelets<sup>145</sup> have been described. Abnormalities in collagen adhesion may be helpful in differentiating between various defects of the platelet release reaction (Chapter 35).

### Platelet Aggregation

Platelet aggregation may be estimated qualitatively both by microscopic and macroscopic techniques. The most commonly

used quantitative methods employ various aggregometers. In essence, these instruments are photometers modified so as to permit measurement of changes in optical density of a platelet suspension under conditions of constant temperature and continuous agitation (Fig. 33-4). Most instruments measure a combination of light scatter and absorption, and modifications which permit simultaneous nephelometric and photometric measurements have been developed.<sup>21,91a</sup> Platelet aggregation usually is studied in suspensions of citrated platelet-rich plasma. Techniques for the study of platelet aggregation in whole blood<sup>5a</sup> and of washed platelet suspensions also have been described.<sup>4</sup>

Adenosine diphosphate (ADP) in 5  $\mu$ M or higher concentrations produces platelet aggregation directly, and is independent of the release of platelet-contained ADP.<sup>66</sup> Various other aggregating agents act mainly by inducing the release of platelet-contained ADP, eg, a suspension of connective tissue particles ("collagen"),<sup>150</sup> epinephrine and

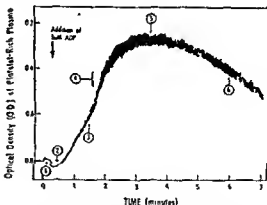


Fig. 33-4. The interpretation of aggregometer tracings. Tracing of platelet aggregation produced by a low concentration of ADP, illustrating normal changes in optical density (OD), ie, (1) a slight decrease due to dilution with aggregating agent, (2) a transient increase due to initial platelet swelling or shape change, (3) a rapid progressive decrease as platelet aggregates form, the size of which is roughly proportional to the amplitude of the oscillations in the tracing (4). The OD then reaches a nadir (5) from which maximal aggregation as a percentage of the initial OD may be calculated from the equation: maximal aggregation (%) =  $OD \text{ at } T_0 - \text{minimum } OD / OD \text{ at } T_0$ . Following this (6) a slow increase in OD due to disaggregation occurs under some conditions.

norepinephrine, and thrombin.<sup>149</sup> With epinephrine (5  $\mu$ M) a weak direct aggregating effect usually can be clearly delineated from the subsequent release reaction which produces a "secondary" wave of aggregation (Fig. 35-2, page 1125). Such "primary" and "secondary" waves of aggregation also may be seen with carefully titrated amounts of ADP (0.2 to 1.5  $\mu$ M).<sup>66</sup> Measurements of released ADP<sup>146</sup> and <sup>14</sup>C-labeled serotonin<sup>149</sup> provide more direct indices of the release reaction (page 394).

### Tests for Platelet Factor 3 Activity

Although the exact nature and significance of platelet factor 3 (PF-3) remain uncertain and controversial,<sup>83,84</sup> most data would suggest that the evolution of this procoagulant activity normally is intimately related to and dependent upon the process of platelet aggregation<sup>127</sup> (Chapter 9). Consequently, estimates of PF-3 activity provide mainly an indirect means of assessing these earlier steps. Despite the widespread use of the term "PF-3 release," it is probable that the activity which is measured by most techniques remains closely associated with the platelet membrane, having been activated or made "available" by various factors, eg, contact with foreign surfaces, ADP, thrombin (page 397). Techniques for the bioassay of PF-3 remain imperfect, despite the large number and variety of methods which have been tried.<sup>83,84</sup> Attempts to measure platelet factor 3 by chemical methods have thus far been unsuccessful.

Platelet suspensions may be prepared, washed, and tested for PF-3 activity in the thromboplastin generation test (TGT). Platelet concentration may be standardized by enumeration, nephelometry,<sup>23,24</sup> or by packed volume.<sup>51</sup> The major disadvantage of this technique is that when platelets are centrifuged into a "button" and washed, PF-3 is made "available" and may be removed and lost as a result. Another significant problem is that dilute platelet suspensions must be used in order to demonstrate an abnormality,<sup>51,84</sup> and the resulting long sub-

strate clotting times are quite variable even with normal platelets.<sup>84</sup> The coagulant activity of a platelet suspension following disruption of the cells by freezing and thawing or sonication is often assumed to be proportional to the total "content" of platelet factor 3. The diagnostic significance of this measurement is nevertheless unclear (page 397).<sup>84</sup>

Tests of kaolin-induced PF-3 availability<sup>63</sup> are more sensitive and reproducible than those based on the TGT. Both the activating surface and platelet concentration can be accurately standardized, and since the test is carried out in platelet-rich plasma, platelet manipulation prior to testing is minimized. In the simplest one-stage method,<sup>64</sup> kaolin and platelet-rich plasma are incubated, with mixing, for a time, and the mixture is then recalcified. The clotting time of such a mixture generally is proportional to the platelet factor 3 made "available" by kaolin. Nonspecific variations may be minimized by two-stage modifications.<sup>126,142</sup>

The one-stage serum prothrombin time, discussed below, and other estimates of prothrombin consumption still are widely used as screening procedures for PF-3 activity, mainly because they are simple to perform. If abnormal as the result of platelet dysfunction, prothrombin consumption will usually be corrected by the addition of small amounts of a platelet substitute.<sup>142</sup> The diagnostic significance of this phenomenon is uncertain.<sup>84</sup>

### Clot Retraction

Clot retraction is usually deficient when the platelet count is below  $50.0 \times 10^3/\mu\text{L}$ <sup>55</sup> and in a rare disorder of platelet function (thrombasthenia<sup>65</sup>, page 1119). It is normal in most other disorders of platelet function. Qualitative estimates of clot retraction can be made by incubating a tube of clotted blood, in which retraction is normally apparent within two hours. Simple quantitative methods for determining the extent of clot retraction have been described<sup>24</sup>; these are useful in the study of disordered platelet function and drug-induced autoimmune thrombocytopenias (page 1092).

### Tests of the Coagulation Phase

It is doubtful whether many of the innumerable minor technical variations which have found their way into methods for the study of blood coagulation have significantly improved the specificity or reproducibility of the commonly used tests. However, the "normal" range of almost every test will vary depending on the exact technique and reagents employed, and it is thus very important that the physician be aware of the normal range for the specific technique employed in a particular laboratory. In general, a meticulous method of performing the test is more important than the exact technique employed.

Blood samples obtained by means of traumatic venipunctures or from indwelling catheters<sup>76</sup> often are inadequate for coagulation studies. Even the small amounts of heparin used to flush indwelling catheters can produce marked coagulation abnormalities. A poorly collected blood sample is a far more common cause of inaccurate results than is technical error, and confusion and delay usually result when coagulation tests are performed on inadequate specimens.

#### *The Partial Thromboplastin Time*

The partial thromboplastin time (PTT) is a simple test of the intrinsic and common pathways of coagulation. When a mixture of plasma and a phospholipid platelet substitute is recalcified, fibrin forms at a normal rate only if the factors involved in the intrinsic pathway (factors XII, XI, IX, and VIII) and the common pathway (factors X, V, prothrombin, and fibrinogen) are present in normal amounts (Fig. 33-5). Platelet substitutes of various kinds may be used, eg, chloroform extract of brain<sup>5</sup> and other crude cephalin fractions, soybean phosphatides (Inosithin). In the PTT, such platelet substitutes are provided in excess, and the test is thus unaffected by platelets. However, platelet substitutes are only "partial" thromboplastins, and they are incapable of activating the extrinsic pathway, which requires "complete" tissue thrombo-

plastins. Thus, the PTT "bypasses" the extrinsic pathway and is unaffected by factor VII.

The PTT is somewhat more sensitive to deficiencies of factors VIII and IX than to factors XI and XII or factors involved in the common pathway,<sup>56</sup> but with most techniques the test will usually yield abnormal results if the plasma level of any of the essential factors is below 15 to 20% of normal. The PTT thus detects many mildly affected patients. The PTT also is prolonged by heparin and by inhibitors of any of the essential factors. As is the case with all one-stage tests, the PTT may be shortened by high levels of a single factor, most commonly factor VIII (page 421). Thus, a short PTT may signify any of the various "hypercoagulable" states (Chapter 39), and high levels of any factor involved may mask deficiencies of others.<sup>49</sup>

In the original method,<sup>79</sup> contact activation was provided by the glass tube, but the addition of "activators," such as ellagic acid, or particulate silicates such as Celite or kaolin, provides more optimal and standardized contact activation, and represents a significant improvement over the original nonactivated test.<sup>110</sup>

#### *The Plasma Prothrombin Time*

The production of fibrin via the extrinsic and common pathways (Fig. 33-5) requires tissue thromboplastin and factor VII, in addition to factors X and V, prothrombin, and fibrinogen. These pathways are measured by the plasma prothrombin time,<sup>111</sup> in which plasma is recalcified in the presence of excess tissue thromboplastin. This test does not require contact activation, and "bypasses" the intrinsic pathway and the factors concerned there, ie, factors XII, XI, IX, and VIII. Since tissue thromboplastins contain phospholipids which act as platelet substitutes, the test is unaffected by platelets. Of the five coagulation factors measured in the prothrombin time, ie, factors V, VII, and X, prothrombin and fibrinogen, three (prothrombin, factors VII and X) are vitamin K-dependent and are depressed by coumarin-like drugs. As

a result, the prothrombin time is the most widely used test for controlling anticoagulant therapy with such drugs (page 1244). The prothrombin time usually will be prolonged if the plasma levels of any of the requisite factors are below 10% of normal, and is relatively more sensitive to deficiencies of factors VII and X than to deficiencies of fibrinogen and prothrombin.<sup>9</sup> The prothrombin time also is prolonged by inhibitors of any of the essential factors, and by heparin, but it is much less sensitive to the anticoagulant action of heparin than is the PTT.

### Related Methods

Various modified techniques and thromboplastins have been developed to improve the utility of the prothrombin time in the control of coumarin anticoagulants<sup>104,105</sup> (Chapter 39). The expression of the prothrombin time as a "percentage" of normal may be misleading and is not recommended, since the dilution curves used to arrive at this figure often have little quantitative meaning. The prothrombin time performed with bovine brain thromboplastin or Thrombotest<sup>104</sup> is abnormal in patients with certain of the genetic variants of factor IX deficiency, but is normal in patients having the more common form of this disorder (page 1172). The venom of Russell's viper contains a unique thromboplastic substance which initiates coagulation by the direct activation of factor X, and does not require factor VII (page 425). The one-stage "prothrombin time" performed with this venom (the Stypven time)<sup>62</sup> thus serves to distinguish between deficiencies of factor VII and those of factor X (Fig. 33-4).

### The Coagulation Time

The coagulation time of whole blood is the most commonly misinterpreted coagulation test. In theory, fibrin formation in whole blood collected without contaminating-tissue thromboplastin proceeds via the intrinsic and common pathways, involves the same coagulation factors measured by the PTT, and requires factor 3 from the contained

platelets (Fig. 33-5). However, the coagulation time measures only the time required for the formation of the first traces of thrombin which suffice to produce a visible clot. Even the small amounts of platelet factor 3 which are available in severe thrombocytopenia are sufficient to produce these requisite traces of thrombin, and consequently the clotting time is unaffected by the number of platelets. For the same reason, the clotting time is significantly prolonged only in severe deficiencies of the various coagulation factors involved in the intrinsic and common pathways, and usually is normal when these factors are present in amounts which exceed 1% of normal plasma levels.<sup>123</sup> The test is unaffected by the level of factor VII. The coagulation time is thus informative only if it is significantly prolonged, and is a very poor screening test which seldom provides information not obtained from the PTT. It is prolonged by heparin, and is still widely used to control the use of this drug, but the PTT appears to be a more convenient and reproducible method (Chapter 39).

### Related Methods

The coagulation time of whole blood in plastic or silicone-coated tubes, and the recalcification time of platelet-poor plasma represent refinements of the glass tube whole blood coagulation time. Although somewhat more sensitive, these techniques do not alter any of the intrinsic limitations discussed.

### The Thromboplastin Generation Test (TGT)

In principle, this two-stage test<sup>7,10</sup> measures the amount and the rate of formation of prothrombinase via the intrinsic pathway (Fig. 33-5). The essential factors are provided by three reagents (Table 10-2, page 411): (1) *serum*, which contains all factors not consumed during coagulation (factors XII, XI, IX, and X); (2) *adsorbed plasma*, which contains factors V and VIII, and variable amounts of factors XII and XI, but no factor X or IX; and (3) *a source of platelet factor*

3, which may be a suspension of the patient's platelets or a platelet substitute. Both serum and the plasma are activated by glass contact and by dilution during preparation of the reagents.

The test is performed in two stages. In the first stage, prothrombinase formation is initiated by the addition of  $\text{Ca}^{++}$  to a mixture of the three reagents (reaction mixture). In the second stage, the prothrombinase formed is assayed by adding a portion of the reaction mixture to normal plasma (substrate plasma) which contains normal amounts of prothrombin and fibrinogen. The prothrombinase content of the reaction mixture, serially determined in this manner, increases with time, and within three to five minutes reaches a maximal level (substrate clotting time from 8 to 15 seconds).

If prothrombinase formation is deficient,

the abnormality can be localized to one or more of the three reagents by substituting control reagents. Results of the test (Table 33-2 and Fig. 33-6) usually are expressed in terms of the reagent found to be abnormal, i.e., serum "defects" (presumptive for deficiencies of factor IX or factor X) or plasma "defects" (presumptive for deficiencies of factor V or factor VIII). In the case of factors XI and XII, an abnormality may be present in both plasma and serum, or in serum alone, and usually is most prominent when both reagents are derived from the patient. The TGT usually gives abnormal results if the level of any essential factor is below 10% of normal,<sup>61</sup> and is thus less sensitive than the PTT in detecting the mildly affected patient. The factor V which is normally adsorbed by platelets (platelet factor 1) may minimize or normalize the plasma "defect" seen in

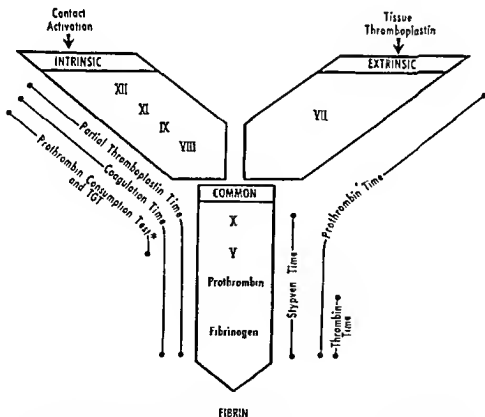


Fig 33 5 The interpretation of common screening tests of blood coagulation \* Platelet factor 3 also is required for normal prothrombin consumption

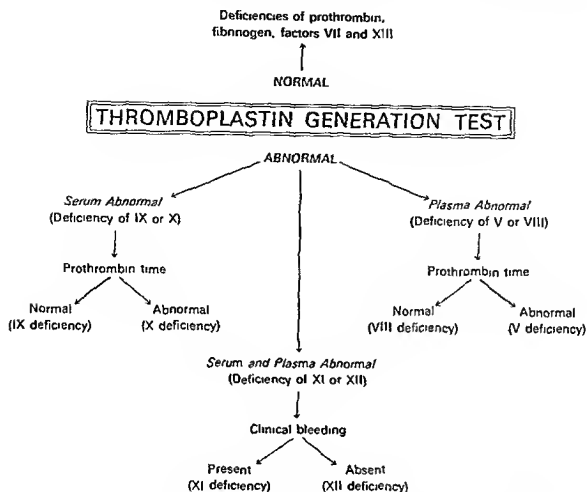


Fig 33-6. The utility of the thromboplastin generation test together with the prothrombin time in the diagnosis of hereditary coagulation disorders. Factor XII or factor XI deficiency may produce either a combined serum and plasma abnormality or a serum abnormality alone.

factor V deficiency if platelets are used rather than artificial substitutes. Abnormal values for TGT may also be caused by various inhibitors of coagulation ("circulating anticoagulants") (Chapter 38). The test is unaffected by deficiencies of prothrombin, fibrinogen, factor VII, and factor XIII.

The TGT can reliably differentiate between the two commonest forms of hemophilia, i.e., hemophilia A and hemophilia B, and does not require plasma from patients with known deficiencies. It is of limited value in the more complex acquired abnormalities of coagulation. Essential reagents are available commercially,<sup>46</sup> but the TGT is still relatively complex and time-consuming. A simplified thromboplastin screening test has been developed.<sup>73</sup>

When the test is used to measure platelet factor 3, platelet suspensions are required, but platelet substitutes save considerable time and are recommended for routine use.

### Related Methods

Although the coagulation time may be normal when only small amounts of prothrombinase are produced, a large amount of unconverted prothrombin will remain in the serum, i.e., prothrombin will not have been normally utilized or consumed. The quantitative measurement of residual prothrombin in serum after a standard interval is thus an indirect measure of the amount of prothrombinase formed, and is the essence of the various prothrombin consumption tests. These



tests measure the same factors as does the TGT, ie, those required for prothrombinase production via the intrinsic pathway (factors XII, XI, IX, VIII, X, and V) (Fig. 33-5). Although more sensitive than the clotting time in that it is abnormal when any of the essential factors are below 2 or 3% of normal,<sup>123</sup> the prothrombin consumption test is less sensitive than either the TGT or the PTT and, like the coagulation time, fails to detect mildly affected patients. Prothrombin conversion is incomplete and the prothrombin consumption test is abnormal in the presence of thrombocytopenia and in certain qualitative abnormalities of the platelets. The two-stage assay for prothrombin and prothrombin consumption<sup>140</sup> is an accurate and specific test, but is elaborate and time-consuming. Various simplified one-stage screening tests for prothrombin consumption have been devised, eg, the serum prothrombin time.<sup>112</sup> These tests may not be truly specific for residual prothrombin,<sup>71</sup> and are uninterpretable in the presence of deficiencies of factor VII or factor X.<sup>102</sup>

Various specialized tests for the factors involved in the contact phase of the intrinsic pathway have been devised, eg, the contact activation test,<sup>87</sup> the celite eluate test, and the celite-6-test<sup>95</sup> which may be valuable in defining an abnormality in the early stages of coagulation when naturally deficient plasmas are not available.

### Assay of Plasma Fibrinogen

Several accurate methods are now available for the quantitative assay of plasma fibrinogen, a measurement of increasing clinical importance which is now available in most laboratories.<sup>57</sup> Fibrinogen usually is converted into fibrin, which is quantitated by gravimetric, nephelometric,<sup>52</sup> or chemical<sup>115</sup> methods. Immunologic<sup>54</sup> and precipitation<sup>109</sup> methods have also been described. Methods which measure coagulable protein<sup>115</sup> are generally the most reliable. Precipitation methods may overestimate the fibrinogen level in intravascular coagulation (DIC),<sup>108</sup> and in the presence of certain anti-

biotics.<sup>93a</sup> Marked differences between the fibrinogen levels obtained by various methods are seen in the hereditary dysfibrinogenemias (Chapter 37).

### Related Methods

In determination of the plasma thrombin time,<sup>62,77</sup> pre-formed thrombin is added to plasma, and the time required for clot formation thus measures the rate at which fibrin is formed (Fig. 33-5). The time is prolonged when the fibrinogen level is below 100 mg/dl, but is unaffected by the levels of any of the other coagulation factors. It is prolonged by heparin and by abnormal amounts of other antithrombins. The thrombin time is technically simple, can be performed quickly, and is valuable, particularly in the diagnosis of DIC.

Screening "kits" for the detection of hypofibrinogenemia are available commercially and are widely used.<sup>122</sup>

### Tests for Intravascular Coagulation and Fibrinolysis ✓

The plasma euglobulin fraction contains plasminogen activators, plasminogen, any active plasmin present, and fibrinogen. Most of the antiplasmins remain in the "pseudoglobulin" supernate. The rate of lysis of a fibrin clot prepared from the euglobulin fraction (the euglobulin clot lysis time)<sup>138</sup> thus provides a means of measuring fibrinolysis in the absence of its inhibitors, and measures mainly the activity of plasminogen activators.<sup>102</sup> It is the most simple and rapid test yet devised for the detection of accelerated fibrinolysis.

Moderate concentrations of epsilon-amino caproic acid (EACA) ( $4 \times 10^{-4}$  M) inhibit plasminogen activators but not free plasmin (Chapter 10). Thus, a shortened euglobulin lysis time in the presence of such concentrations of EACA indicates the presence of free plasmin, eg, in fibrinogenolysis (Chapter 38). Fibrinolysis in heated fibrin plates<sup>3</sup> also measures free plasmin, since plasminogen activators are thermolabile.

The rate of whole blood clot lysis is a gross measurement of fibrinolysis, and its determination requires only the incubation and observation of a blood clot, eg, one of the samples obtained for a clotting time. If very rapid, whole blood clot lysis may be of diagnostic significance, but otherwise the time required for lysis, which normally is in excess of 24 hours, is usually too long to be of diagnostic help. Neither the euglobulin clot lysis time nor the whole clot lysis time is interpretable in the presence of severe hypofibrinogenemia, and may be normal in DIC as a result of plasminogen depletion.

The serial thrombin time provides an indirect assessment of the proteolytic effects of plasmin, but it is also abnormal in hypofibrinogenemia and in the presence of antithrombins such as fibrin degradation products.<sup>32</sup>

Tests for fibrinolysis and assays for individual components of the fibrinolytic enzyme system, including plasminogen activators, plasminogen, free plasmin, and antiplasmins, have been perfected<sup>130</sup> (page 432). These are largely research tools, but may be of diagnostic value in some cases of DIC and fibrinolysis.

Fibrin-fibrinogen degradation products (FDP) are protein fragments of various sizes which result from the proteolytic action of plasmin on fibrin or fibrinogen (Fig. 10-8, page 437). They are commonly present in DIC and fibrinolysis and are of considerable diagnostic significance. Quantitative assays for these fragments based on techniques such as red cell hemagglutination inhibition,<sup>89</sup> staphylococcal agglutination,<sup>80</sup> and immunodiffusion<sup>114,132</sup> (page 436) have been described. Most of these methods are rather time-consuming. Tests for FDP employing commercial reagents have also been described.<sup>89,114</sup>

Certain FDP are potent antithrombins which may prolong the thrombin time even when the fibrinogen level is above 100 mg/dl. The thrombin time obtained on a mixture of equal parts of normal plasma and patient's plasma will be normalized if the prolongation is due to fibrinogen deficiency,

but will remain abnormal if due to the presence of antithrombins such as FDP. This modification of the thrombin time ("corrected" thrombin time)<sup>24,62</sup> may thus be used as an indirect indicator of the presence of FDP; while quite nonspecific, the test is valuable primarily because it can be performed quickly.

Unpolymerized fibrin monomers (page 429) are commonly present in the blood in DIC. Various techniques ("paracoagulation" techniques) for demonstrating such monomers have been described. These range from the ethanol gelation test,<sup>28</sup> which is relatively insensitive, to various protamine gelation techniques,<sup>58,95,120</sup> which are highly sensitive but relatively nonspecific.<sup>44</sup> Of apparently similar significance are "cryofibrinogens" (page 1221) which may be demonstrated in some cases of DIC.

### Bioassays for Coagulation Factors

Bioassays for coagulation factors are usually based on the familiar screening and confirmatory tests, eg, the prothrombin time, partial thromboplastin time, and thromboplastin generation test. In principle, the extent to which an unknown sample corrects the abnormality in a plasma with a known deficiency is assumed to be proportional to the content of the deficient factor in the sample. The results are usually expressed as "percent of normal" which, depending on the exact method employed, normally ranges from 50 to 200%. The limitations and variables involved in such bioassay systems are considerable and have been discussed at length.<sup>10,62,75,81,116</sup>

Although there is no general agreement regarding the relative merits of one- and two-stage methods,<sup>6</sup> one-stage methods for factors VIII and IX which are based on the partial thromboplastin time and employ substrate plasma from patients with severe hereditary deficiencies of these factors have proved satisfactory in most laboratories, and are somewhat simpler to perform than comparable two-stage methods based on the thromboplastin generation test. However, the

availability of deficient plasma from patients often poses a major problem, and, as a consequence, methods have been developed which do not depend on natural substrates. These include techniques for the assay of prothrombin,<sup>106,140</sup> factors V,<sup>62,78</sup> VII and X combined,<sup>105</sup> and factors VIII,<sup>53</sup> IX,<sup>129</sup> X,<sup>42</sup> and XI.<sup>74</sup>

### *Tests for Inhibitors of Coagulation*

#### *(the "Circulating Anticoagulants")*

Abnormalities in any test of coagulation, if due to deficiency of an essential factor, will be corrected by the addition of small amounts of normal plasma or blood. If the abnormality is due to one of the various inhibitors of coagulation rather than a deficiency, the opposite is true, ie, small amounts of the patient's plasma will impair coagulation in normal samples. This is the essence of all tests for inhibitors, most of which are based on common screening tests, eg, the plasma recalcification time, the partial thromboplastin time,<sup>62</sup> the thromboplastin generation test.<sup>10,62</sup> The test system must often be adapted to the particular type of inhibitor (page 1208). The presence of exogenous heparin may be confirmed by means of various protamine neutralization tests.<sup>24,62</sup>

### *Automated Methods*

A variety of instruments have been developed which automatically detect the end point clotting time, and are helpful in the performance of the one-stage screening tests, eg, the prothrombin time and the partial thromboplastin time.<sup>91,122a,137</sup> Totally automated methods for performing these tests are being evaluated.<sup>2</sup>

The thromboelastograph is an instrument that demonstrates changes that occur during blood coagulation and fibrinolysis, and has been used extensively by some investigators. Different "coagulograms" are described in association with various bleeding disorders and hypercoagulable states.<sup>41,102</sup>

## **Initial Laboratory Approach**

### **The Utility of Screening Tests**

The initial laboratory study of the bleeding patient should be guided by the information obtained from the clinical evaluation. However, the routine use of a small battery of screening tests has merit in many cases, since it serves to direct the course of further study and usually saves time. It is generally agreed that the most essential information can usually be obtained from four tests summarized in Table 33-3, which, in view of their availability, simplicity, and low cost, are admirably suited to serve as "primary" screening tests. The platelet count and the bleeding time together provide the most reliable and reproducible tests of the vascular and platelet phases. The partial thromboplastin time measures all of the coagulation factors involved in the intrinsic and common pathways (Fig. 33-5) and is generally accepted as the best single screening test for disorders of blood coagulation. When supplemented with the plasma prothrombin time, which assesses the extrinsic as well as the common pathway, the abnormality can usually be localized to one of the three pathways and the factors involved therein (Fig. 33-5 and Table 33-3). Together, these four tests will thus provide a "presumptive diagnosis." This can then be further clarified by the confirmatory methods summarized in the following section. The definitive laboratory diagnosis of individual bleeding disorders is covered in Chapters 34 to 39, inclusive.

### **Confirmatory Tests**

#### **Thrombocytopenia**

Thrombocytopenia, like anemia, is a symptom and not a diagnosis. It is the most common of the acquired platelet disorders whereas hereditary forms are rare. Additional laboratory tests usually are not indicated merely to confirm the presence of thrombocytopenia (Table 33-3, A). The bleeding time, clot retraction, and the results of the

Table 33-3. The Presumptive Diagnosis of Common Bleeding Disorders by Means of

Platelet Count	Bleeding Time	Partial Thromboplastin Time	Prothrombin Time	"Presumptive Diagnosis"	Common Causes	
					Hereditary	Acquired
A Decreased	Prolonged	Normal	Normal	Thrombocytopenia	Aldrich syndrome (p 1127)	ITP (p 1075) drugs (p 1092) other secondary forms
B Normal or elevated	Prolonged	Normal	Normal	Disorder of platelet function	Thrombasthenia (p 1119) Deficient release reaction (p 1123)	Drugs (p 1128), uremia (p 1129) dysproteinemias (p 1130) thrombocythemia (p 1103)
C Normal	Prolonged	Prolonged	Normal	von Willebrand's disease (p 1179)		
D Normal	Normal	Prolonged	Normal	Coagulation abnormality in intrinsic pathway	Hemophilia A (p 1161) or B (p 1172) Deficiency of factors XI (p 1173) and XII (p 1179)	Inhibitors (p 1208)
E Normal*	Normal	Prolonged	Prolonged	Coagulation abnormality in common or multiple pathways	Deficiency of factors V, X (p 1178) prothrombin and fibrinogen dysfibrinogenemias (p 1174)	Liver disease (p 1205), vitamin K deficiency (p 1201), intravascular coagulation (p 1211), fibrinogenolysis (p 1224)
F Normal	Normal	Normal	Prolonged	Coagulation abnormality in extrinsic pathway	Deficiency of factor VII (p 1178)	
G Normal	Normal	Normal	Normal		Deficiency of factor XIII (p 1176) Telangiectasia (p 1144)	Allergic purpura (p 1136), scurvy (p 1150), drugs (p 1142), autoerythrocyte sensitization (p 1151)

\*May be abnormal in acquired disorders which produce deficiencies of multiple coagulation factors

prothrombin consumption test and the tourniquet test will yield abnormal values in the presence of significant thrombocytopenia. However, none of these tests is as accurate or reproducible as the platelet count, nor do they correlate any better than does the platelet count with the severity of bleeding in patients with thrombocytopenia. The differential diagnosis of thrombocytopenia is discussed at length in Chapter 34.

### Qualitative Disorders of Platelet Function

A prolonged bleeding time in the presence of a normal number of platelets and normal coagulation tests (Table 33-3, B) is presumptive evidence of a disorder of platelet function. Hereditary forms are uncommon, but bleeding due to platelet dysfunction may be an important complication in patients with various acquired disorders, eg, uremia. Commonly available confirmatory tests provide little ancillary information regarding platelet function. Tests of prothrombin consumption frequently yield abnormal values as a consequence of deficient activity of platelet factor 3. Deficient clot retraction suggests thrombasthenia, a very rare disorder. Various morphologic abnormalities of the platelets may be seen. Strikingly large platelets are characteristic of certain hereditary disorders, eg, the Bernard-Soulier syndrome (page 1126). A markedly elevated platelet count suggests hemorrhagic thrombocythemia and related disorders (page 1103).

Definitive methods for the study of these disorders are very time-consuming and technically difficult (Chapter 33). The absence of platelet aggregation by ADP, collagen, and epinephrine is characteristic of thrombasthenia (page 1119). Deficient collagen and epinephrine-induced aggregation despite normal ADP-induced aggregation are found in association with various disorders of the release reaction, eg, hereditary deficiency of storage nucleotides, uremia, aspirin ingestion. Disordered platelet function is discussed in Chapter 35.

### von Willabrand's Disease

This disorder is the result of an abnormality in "primary" hemostasis combined with mild to moderate deficiency of factor VIII (Chapter 37). In the typical case, the "primary" screening tests reveal a prolonged bleeding time and a prolonged partial thromboplastin time (Table 33-3, C). In many of these patients, only the bleeding time is abnormal, and in others there may be no detectable abnormalities. Furthermore, the results of all of the laboratory tests tend to fluctuate from time to time. The coagulation time and the results of the prothrombin consumption test may be abnormal if factor VIII deficiency is severe, but usually are normal. Useful confirmatory tests include measurements of ristocetin-induced platelet aggregation, in vitro platelet retention in glass-bead columns, and demonstration of "new" factor VIII synthesis following plasma infusions (page 1180). The last-named test is difficult to reproduce and is cumbersome.<sup>113</sup> In many cases, the diagnosis depends on repeated observations over a period of time.

### Disorders of the Intrinsic Pathway of Coagulation

Disorders characterized by a prolonged partial thromboplastin time and a normal prothrombin time (Table 33-3, D) include hemophilia A and hemophilia B, and deficiencies of factors XI and XII. These together comprise over 80% of all hereditary coagulation disorders (Chapter 37). Factor XII (Hageman factor) deficiency can be readily excluded since it is not associated with bleeding. The thromboplastin generation test provides the most simple way to distinguish between the remaining three, and, together with the prothrombin time, affords what is essentially a specific diagnosis (Fig. 33-5). The coagulation time and results of the prothrombin consumption test depend on the severity of the deficiency, and should never be relied upon to exclude these disorders

since the results of both tests will be abnormal only in severely affected patients.

In deficiencies of factors XI, XII, and possibly Fletcher factor deficiency (page 1183), the results of the activated partial thromboplastin time may be the same as those obtained with the nonactivated method, and, when silicone-coated tubes are used, the coagulation time and the results of the prothrombin consumption test may be normal, in contrast to the gross abnormalities found when glass tubes are used.

Acquired coagulation disorders are seldom associated with a prolonged PTT and a normal prothrombin time. Important exceptions are the various inhibitors or circulating anticoagulants (Chapter 38). A slight prolongation of the partial thromboplastin time together with a normal prothrombin time is frequently the result of a poorly collected blood sample, and is not uncommon in patients with liver disease.<sup>130</sup>

### Disorders of the Common Pathway of Coagulation

In a patient with a hereditary disorder, prolongation of the PTT and the prothrombin time indicates a deficiency of one of the factors in the common pathway, ie, either factor X, factor V, prothrombin, or fibrinogen (Table 33-3, E). Such isolated deficiencies are exceedingly rare. On the other hand, in many of the most common acquired coagulation disorders, deficiencies of one or more of these factors are associated with additional abnormalities in the intrinsic and extrinsic pathways, eg, vitamin K deficiency, intravascular coagulation. As a consequence, a prolonged prothrombin time always suggests an acquired disorder, and usually is associated with a complex abnormality involving multiple pathways, eg, DIC.

When confronted with this combination of findings, the first step should be to exclude or identify an abnormality in the thrombin-fibrinogen reaction (Fig. 33-7). This may be accomplished by determination of the thrombin time and fibrinogen assays. The most helpful ancillary procedures are the platelet

count, examination of the blood smear for schistocytes, the thrombin time, the fibrinogen level, the euglobulin lysis time, and tests for FDP. The laboratory diagnosis of DIC is summarized in Chapter 38.

Hereditary disorders which are associated with a prolonged thrombin time and low fibrinogen levels include hereditary afibrinogenemia, hypofibrinogenemia, and dysfibrinogenemia (page 1174).

Hereditary deficiencies of factor V, factor X, and prothrombin can be distinguished from one another by simple correction tests<sup>37,102</sup> and by the thromboplastin generation test (Figs. 33-6 and 33-7).

### Disorders of the Extrinsic Pathway of Coagulation

A prolonged prothrombin time and a normal partial thromboplastin time (Table 33-3, F) suggest an isolated deficiency of factor VII, which is very rare. Since factor VII is essential only in the tissue-activated extrinsic pathway of coagulation, the coagulation time, the Stypven time, and thromboplastin generation are normal in this disorder.

### Disorders in Which Results of "Primary" Screening Tests Are Normal

The screening tests usually yield normal results in patients with bleeding disorders due to vascular abnormalities (Tables 33-3, G and 33-4). Laboratory techniques for the study of these disorders are crude or nonexistent. A slight prolongation of the bleeding time or a positive reaction to the tourniquet test may be obtained in some cases, but the diagnosis usually is made from the associated clinical findings which are often characteristic, eg, the skin lesions of hereditary hemorrhagic telangiectasia, allergic purpura, scurvy, senile purpura. A positive reaction to a specific skin test may confirm the diagnosis of autoerythrocyte sensitization and related disorders (Chapter 36). The results of screening tests also are normal in factor XIII (fibrin stabilizing factor) deficiency (Chapter 37), a disorder in which the diagnosis is made by the demon-

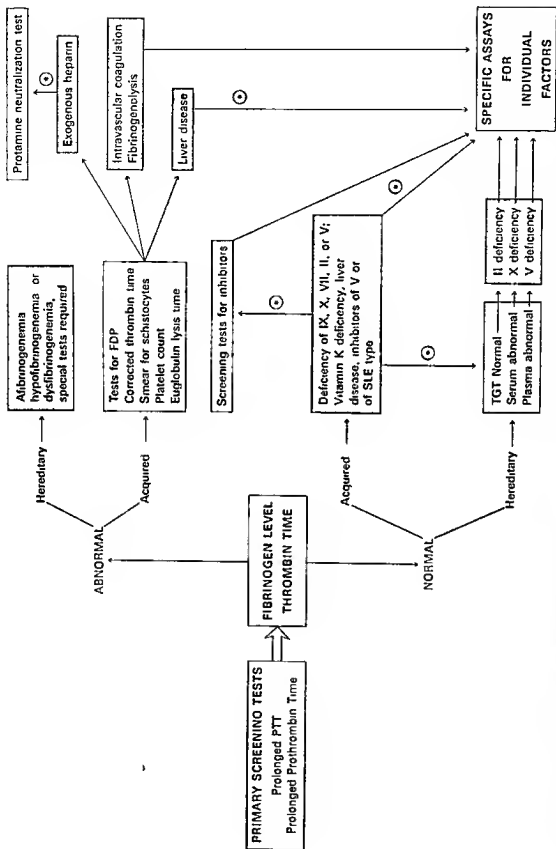


Fig. 33-7. Flow sheet for laboratory evaluation of coagulation disorders of common or multiple pathways. Tests are indicated in solid blocks; presumptive diagnoses in shaded blocks. Only the most common disorders are included. Asterisks denote disorders in which the clinical picture is usually clear-cut and in which the ancillary laboratory studies indicated by arrows are seldom required.

stration of characteristic clot solubility in urea or other protein solvents.<sup>62</sup>

Although abnormal in the typical case, the results of screening tests may be normal or equivocal in patients with mild coagulation disorders, including heterozygous carriers, in patients with certain disorders of platelet function, and in those with mild forms of von Willebrand's disease. Various as yet undefined abnormalities of the contact phase of coagulation also may not be detected in the "primary" screening tests.

Patients with a significant bleeding history, in whom the results of detailed studies of hemostasis and blood coagulation are normal, constitute a larger problem. Such "bleeders" are not uncommon, and probably suffer from disorders of hemostasis which cannot be detected by presently available methods. These patients should always be managed with great care, and it should be emphasized that a clear-cut history of bleeding is always more significant than negative laboratory data.

Trauma may be denied in the "battered-child" or "battered-wife" syndrome, and may be self-inflicted in psychotic or neurotic patients.

The value of routine screening tests prior to surgical procedures has been debated for years. It is clear that the coagulation time, estimates of clot retraction, whole clot lysis time, and the tourniquet test are of little value, and very few abnormalities will be

detected by even the more sensitive screening methods in patients with a completely negative history and normal findings on physical examination. To the contrary, "routine" pre-operative laboratory screening is of great value in certain "high-risk" patients who suffer from disorders which are known to predispose to unexpected postsurgical bleeding,<sup>38</sup> even from relatively limited biopsy procedures. Important in this category are patients with liver disease, biliary obstruction, renal disease particularly if complicated by azotemia, myelofibrosis, and those with the dysproteinemias. To this list should be added all patients scheduled to undergo procedures involving the use of extracorporeal circulatory devices.

## Laboratory Evaluation in the Newborn

Laboratory investigation of hemostasis and blood coagulation in the neonate and infant differs from that outlined above in several respects. Firstly, the quantity of blood which can be obtained is limited, and the venipuncture is frequently difficult. Various micro-techniques, and methods which employ capillary blood<sup>40b,47,62,66,93</sup> have been devised to circumvent this problem. Secondly, in terms of adult norms, the results of most tests vary widely and often are abnormal even in healthy full-term infants. This presumably is the result of deficiencies of the vitamin K-dependent factors, and additional "physiologic" abnormalities which have been demonstrated in the contact phase and in the thrombin-fibrinogen reaction.<sup>1,101</sup> The prothrombin time may be prolonged, but is often normal if vitamin K is administered to the infant or mother.<sup>1</sup> Abnormalities of the thrombin time, the partial thromboplastin time, and in the results of thromboplastin generation test are common, and may persist for months.<sup>101</sup> These abnormalities are more pronounced in the premature than in the full-term infant, and in extent are inversely proportional to gestational age and birth weight.<sup>26</sup> The levels of fibrinogen and factors V and VIII are usually normal.<sup>1</sup> Tests for

**Table 33-4. Disorders in Which the Results of the "Primary" Screening Tests May Be Normal**

- 1 von Willebrand's disease
- 2 Mild hereditary coagulation disorders, particularly factor XI deficiency
- 3 Heterozygous carriers of hereditary coagulation disorders
- 4 Factor XIII (fibrin stabilizing factor) deficiency
- 5 Mild, as yet undefined, abnormalities in the contact stage of coagulation
- 6 Some forms of dysfibrinogenemia
7. Disordered platelet function particularly deficient release reaction
- 8 Hereditary hemorrhagic telangiectasia
- 9 Allergic and "vascular" purpuras ✓



FDP and the euglobulin lysis time may be unreliable if carried out on cord blood. Significant abnormalities of platelet aggregation and of other platelet function tests may be seen in normal neonates,<sup>94</sup> but the platelet count in term infants as well as in thriving prematures approaches adult norms.

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## *Quantitative Variations of Platelets in Disease; Thrombocytopenia and Thrombocytosis*

### Thrombocytopenia

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### Thrombocytosis

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## Thrombocytopenia

Purpura in association with pestilential fevers was described by Hippocrates and later writers, but it was not until the 16th century (Lusitanus) and the early part of the 17th century (La Rivière) that purpura in the absence of fever was recognized.<sup>57</sup> Werlhof in 1735 distinguished "morbus maculosus hemorrhagicus" as a separate entity, and Willan in 1808 classified purpura under the headings (1) simplex, (2) haemorrhagica, (3) urticans, and (4) contagiosa, thus separating the types later described by Schönlein (1829) and Henoch (1868) which are now known by their names (Chapter 36). The marked diminution in "hematoblasts" (platelets) in "purpura hemorrhagica" was recognized by Krauss (1883) and by Denys (1887). Hayem (1895) noted the non-retractility of the blood clot, and Duke (1912) demonstrated the prolonged bleeding time. Abnormal capillary fragility was observed by writers in different countries ("le signe du lacet," Grocco-Frugoni's sign, the Rumpel-Leede phenomenon, Hess' capillary resistance test).<sup>51</sup>

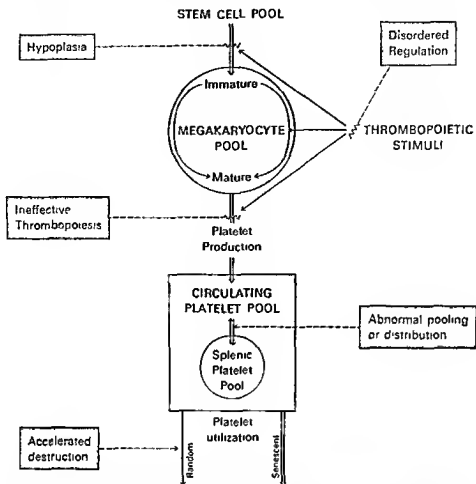


Fig 34-1 The pathophysiology of thrombocytopenia. A simplified diagram of the biodynamics of the megakaryocyte-platelet system is indicated in solid lines. The mechanisms by which pathologic processes (shaded blocks) produce thrombocytopenia are indicated in dashed lines.

### Pathophysiology

Thrombocytopenia may be defined as a subnormal number of platelets in the circulating blood. It is the most common cause of abnormal bleeding. The pathophysiology of thrombocytopenia is quite similar to that of anemia, but the latter is better understood. Thus, despite the number and diversity of disorders that may be associated etiologically, thrombocytopenia results from only three processes. These are: (1) deficient platelet production; (2) accelerated platelet destruction; and (3) abnormal distribution or pooling of the platelets within the body (Fig. 34-1).<sup>395</sup> The changes in the basic parameters

of thrombopoiesis that are characteristic of each of these processes are summarized in Table 34-1.

*Accelerated platelet destruction* is the commonest cause of thrombocytopenia. It leads to stimulation of thrombopoiesis, and consequently to an increase in the number, size, and rate of maturation of the precursor megakaryocytes (Fig. 34-1). When the rate of platelet destruction exceeds this compensatory increase in platelet production, thrombocytopenia develops. "Compensated" platelet destruction without thrombocytopenia may also occur,<sup>397a</sup> eg, in patients with prosthetic heart valves<sup>415</sup> and in patients with ITP following splenectomy.<sup>45</sup>

Table 34-1. Thrombokinetetic Patterns in Various Forms of Thrombocytopenia

Measurement	Decreased Production			
	Hypoproliferation or Hypoplasia*	Ineffective Thrombopoiesis†	Accelerated Destruction‡	Abnormal Pooling
Total megakaryocyte mass§	Decreased	Increased	M increased	Var increased
Megakaryocyte number	Decreased	M increased	Increased	Var increased
Megakaryocyte volume	Increased	Normal or Var decreased	Increased	Var increased
Platelet turnover rate or production rate	Decreased	Decreased	Increased	Var increased
Total platelet mass	Decreased	Decreased	Decreased	? Normal
Splenic platelet pool	Decreased	Decreased	Decreased¶	Increased
Platelet survival	Normal	Var shortened	Shortened	Var shortened

KEY Var = variably M = markedly

\*Includes myelophthisic processes

†Mainly in megaloblastic hematopoiesis component of accelerated destruction present in some cases

‡Minor component of "ineffective" thrombopoiesis present in some cases

§Equated to "total" thrombopoiesis

|| Equated to "effective" thrombopoiesis

¶Not representative of sequestered antibody-sensitized platelets

Based on the work of Harker et al.<sup>45,194,195,196</sup> The data upon which this table is based are tentative since techniques for the study of platelet kinetics have been developed only recently

Platelet destruction may result from both "intracorporeal" and "extracorporeal" abnormalities. Intracorporeal defects are rare, but have been demonstrated in certain forms of hereditary thrombocytopenia, eg, the Wiskott-Aldrich syndrome (page 1127).<sup>336,396</sup> In such disorders, the survival of affected platelets is shortened both in the patient and in normal recipients. More frequently, platelet destruction is the result of extracorporeal factors, various immunologic phenomena being the most common. Platelets injured by either intracorporeal or extracorporeal processes usually are removed from the circulation by the spleen, liver, and reticuloendothelial system.

Platelet utilization in intravascular thrombi or on damaged endothelial surfaces is another cause of thrombocytopenia. This occurs in diffuse intravascular coagulation (page 1211), and in thrombotic thrombocytopenic purpura and other microangiopathic processes (Chapter 28).

Deficient platelet production may result from any of a number of processes. Those that depopulate the stem cell or megakaryocyte compartments are the most common, eg, marrow injury by myelosuppressive drugs or irradiation, aplastic anemia (Chapter 56).

Deficient platelet production also may be the consequence of disordered proliferation within a precursor compartment of normal or even increased size. For example, in disorders characterized by megaloblastic hematopoiesis, hypertrophy of the precursor compartment occurs in response to thrombopoietic stimuli, but thrombopoiesis is "ineffective" and platelet production is insufficient. Rarely, abnormalities of those processes that normally regulate thrombopoiesis appear to underlie deficient platelet production, eg, deficiency of "thrombopoietin," cyclic thrombocytopenia (page 1098).

Abnormal pooling or abnormal in vivo distribution of an essentially normal total platelet mass may produce thrombocytopenia. This is seen in the various disorders associated with splenomegaly (Chapter 45), in which platelet production is normal or even increased, but the majority of the platelets are sequestered in the vastly enlarged extravascular splenic pool.

### Classification

All disorders in which purpura was associated with thrombocytopenia were at one time considered to be a single entity, and various

Table 34-2. Etiologic Classification of Thrombocytopenia<sup>a</sup>

## I PRIMARY VARIETIES

A *Idiopathic thrombocytopenic purpura (ITP)*B *Hereditary forms (Table 34-6)*C *Miscellaneous (congenital megakaryocytic thrombocytopenia cyclic thrombocytopenia, tidal platelet dysgenesis deficiency of thrombopoietin)*

## II SECONDARY OR SYMPTOMATIC VARIETIES

A *Chemical or physical agents (Table 34-5)*B *Disorders of the hematopoietic system*1 *Idiopathic aplastic anemia*2 *The myelophthitic processes (acute\* and chronic leukemias metastatic carcinoma\* multiple myeloma and other paraproteinemias histiocytoses myelofibrosis osteopetrosis)*3 *Deficiencies of vitamin B<sub>12</sub> or folic acid (pernicious anemia others)*4 *Severe iron-deficiency anemia*5 *Hemolytic anemias*a *Microangiopathic types (thrombotic thrombocytopenic purpura artificial heart valves others)*b *Paroxysmal nocturnal hemoglobinuria acute autoimmune varieties (Evans syndrome) others*C *Disorders of the spleen*1 *Neoplastic (the lymphomas)*2 *Congestive (portal hypertension vascular anomalies)*3 *Infiltrative (Gaucher's disease, Niemann-Pick disease Letterer-Siwe disease)*4 *Infectious (kala azar miliary tuberculosis syphilis)*5 *Of unknown cause (primary hypersplenism)*D *Infections\**1 *Viral (rubella, rubola<sup>308</sup> measles<sup>290</sup> pertussis<sup>300</sup> herpes simplex<sup>442</sup> mumps<sup>281</sup> infectious mononucleosis<sup>289</sup> 293 small pox,<sup>285</sup> 292 chickenpox,<sup>224</sup> cytomegalic inclusion disease<sup>320</sup> epidemic hemorrhagic**fever, influenza,<sup>285</sup> Colorado tick fever,<sup>297</sup> infectious hepatitis,<sup>223</sup> cat scratch fever,<sup>244</sup> psittacosis, Thai hemorrhagic fever and dengue fever<sup>305</sup> 311)*2 *Rickettsial (typhus,<sup>313</sup> Rocky Mountain spotted fever,<sup>319</sup> others)*3 *Bacterial (septicemia due to gram-negative bacilli,<sup>279</sup> meningococcemia,<sup>322</sup> tuberculosis<sup>146</sup> 295 typhoid fever,<sup>286</sup> subacute bacterial endocarditis,<sup>307</sup> 460 congenital syphilis<sup>283</sup>, 321 brucellosis,<sup>280</sup> scarlet fever<sup>282</sup>, others)*4 *Mycotic (histoplasmosis<sup>288</sup>)*5 *Protozoal (malaria,<sup>276</sup> 287, 317 toxoplasmosis<sup>375</sup>)*6 *Metazoal (ancylostomiasis<sup>458</sup>)*E *Miscellaneous —*1 *Isimmunization (following blood transfusions, fetomaternal incompatibility)*2 *Diffuse intravascular coagulation (DIC) and fibrinogenolysis (Table 38-3, page 1212)*3 *Liver disease\**4 *Uremia*5 *Vascular neoplasms\* (giant hemangioendotheliomas [Kasabach-Merritt syndrome] Kaposi's sarcoma<sup>458</sup> placental chorangiomas<sup>344</sup>)*6 *Extracorporeal circulatory devices\**7 *Massive blood transfusions,\* exchange transfusions*8 *Erythroblastosis fetalis*9 *Heat stroke,<sup>301</sup> insect<sup>390</sup> and snake bites,\* allergy to food,<sup>418</sup> tuberculin and certain vaccines,<sup>315</sup> 406 anaphylactic reactions*10 *Massive burns*11 *Cyanotic congenital heart disease,\*<sup>339</sup> congestive heart failure<sup>445</sup>*12 *Eclampsia<sup>453</sup>*13 *Hyperthyroidism<sup>423</sup> and hypothyroidism<sup>441</sup>*14 *Renal vein thrombosis<sup>404</sup>*<sup>a</sup>May be the result of diffuse intravascular coagulation in some cases

names were applied, such as purpura hemorrhagica, Werlhof's disease, morbus maculosus Werlhofi, hemogenia, hemogenic syndrome,<sup>107</sup> thrombocytolytic purpura. When it was found that thrombocytopenia may result from a large variety of definable causes, thrombocytopenia in such disorders was termed "secondary" or "symptomatic" in contradistinction to "essential," "primary," or "idiopathic" forms. Even though the terms

"primary" and "idiopathic" have no real meaning in modern medicine, this distinction has proved to be clinically useful, and provides the basis for the etiologic classification of thrombocytopenia presented in Table 34-2.

A classification of thrombocytopenia based on the above-described pathophysiologic criteria is presented in Table 34-3. Such a classification must be regarded as tentative, since

**Table 34-3. Pathophysiologic Classification of Thrombocytopenia**

- I DEFICIENT THROMBOPOIESIS ✓
  - A *Hypoplasia or suppression of megakaryocytes*
    - 1 Chemical and physical agents (Table 34-5, Table 56-2, page 1746)
    - 2 Idiopathic aplastic anemia and related disorders (congenital megakaryocytic hypoplasia Fanconi syndrome others)
    - 3 Myelophthisic processes, some viral infections
  - B *Ineffective thrombopoiesis* (disorders due to deficiency of vitamin B<sub>12</sub> or folic acid paroxysmal nocturnal hemoglobinuria some hereditary forms, others)
  - C *Disordered control mechanisms* (deficiency of thrombopoietin, tidal platelet dysgenesis cyclic thrombocytopenia)
  - D *Miscellaneous* (many hereditary forms)
- II ACCELERATED PLATELET DESTRUCTION UTILIZATION OR LOSS
  - A *Due to immunologic processes*
    - 1 Autoantibodies (ITP drug induced antibodies [Table 34-5] various hemolytic anemias, SLE, lymphoreticular disorders hyperthyroidism, others)
    - 2 Isoantibodies (due to fetomaternal incompatibility, post-transfusion)
    - 3 Other immunologic processes (allergies erythroblastosis fetalis anaphylactic reactions, immune complexes)
  - B *Due to nonimmunologic processes*
    - 1 Diffuse intravascular coagulation (Table 38-3, page 1212) Kasabach-Merritt syndrome (giant hemangioendotheliomas), many infections
    - 2 Microangiopathic processes (thrombotic thrombocytopenic purpura, prosthetic cardiac valves, many others)
    - 3 Miscellaneous (some infections, massive transfusions and exchange transfusions, extracorporeal circulatory devices fibrinogenolysis, Ristocetin, some hereditary forms)
- III ABNORMAL PLATELET DISTRIBUTION OR POOLING
  - A *Disorders of the spleen* (neoplastic, congestive, infiltrative, infectious, of unknown cause [Table 45-1, page 1407])
  - B *Hypothermic anesthesia*

presently available methods for studying thrombopoiesis are relatively crude, and in many disorders multiple pathogenetic factors may simultaneously or sequentially play a role in the production of thrombocytopenia.

The following discussion will utilize the most suitable nosologic features of both the etiologic and the pathophysiologic classifications, since neither alone is entirely satisfactory.

## Immunologic Platelet Destruction

### Idiopathic Thrombocytopenic Purpura

#### Definition

The term "idiopathic thrombocytopenic purpura" (ITP) is usually employed to refer to instances of thrombocytopenia associated with no apparent exogenous etiologic factors, underlying diseases known to be associated with "secondary" thrombocytopenia having been excluded. The presence of a normal or increased number of megakaryocytes in the bone marrow has been observed so consistently that this finding is now widely accepted as an additional criterion for the diagnosis of ITP. ITP is thus a diagnosis of exclusion, and must be regarded as a syndrome that may arise in several different ways and one that undoubtedly encompasses a variety of fundamentally different disorders. Proposals to further restrict or broaden these admittedly vague diagnostic criteria, or to redefine the meaning of the term ITP<sup>4</sup> have not been widely accepted. This syndrome has been reviewed in detail.<sup>4,16,40,115,271,421</sup>

There are several differences between *acute ITP* and *chronic ITP*; these are of particular significance in the interpretation of data concerning the incidence of the disorder, its prognosis, and the results of therapy (Table 34-4). The differences may illustrate merely the wide spectrum of disorders that by definition are included in the syndrome, but many would now accept the view that acute ITP and chronic ITP are fundamentally different disorders.<sup>4,40</sup>

#### Incidence

ITP is more common than all secondary forms of thrombocytopenia combined.<sup>371</sup> The disorder occurs most frequently in children and young adults.<sup>112</sup> In 67% of a series



Table 34-4. Features of Acute and Chronic ITP

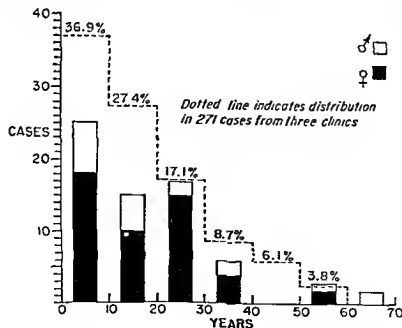
	Acute	Chronic
Peak age incidence	Children 2-6 years of age	Adults, 20-40 years of age
Sex predilection	None	3:1 ratio of female to male cases
Antecedent infection	Common 1-3 weeks prior to onset	Unusual
Onset of bleeding	Abrupt	Insidious
Hemorrhagic bullae in mouth	Present in severe cases	Usually absent
Platelet count	$< 20 \times 10^9/l$	$30-80 \times 10^9/l$
Eosinophilia and lymphocytosis	Common	Rare
Duration	2-6 weeks rarely longer	Months or years
Spontaneous remissions	Occur in 80% of cases	Uncommon; fluctuating course common

of 271 patients,<sup>14,33,137</sup> the disease appeared before the age of 21 years (Fig. 34-2). In a summary of 737 cases reported in the literature,<sup>421</sup> 45% of the patients were found to be 15 years of age or younger. It is well recognized that ITP occurs more frequently in females than in males.<sup>137</sup> The ratio in different series has ranged from 4:3 to 3:1.<sup>100, 125,344</sup> The condition seems to be uncommon among Negroes.<sup>137</sup>

These figures become more intelligible when cases are divided into acute and chronic types (Table 34-4). Thus, acute ITP is com-

monest in children two to six years old<sup>75,89</sup> and rarely may develop in the first year of life.<sup>126</sup> It is uncommon in adults, has no sex predilection, but does occur with a peak incidence during the fall and winter, thus paralleling the prevalence of upper respiratory tract infections.<sup>40</sup> Chronic ITP may be encountered in persons of all ages, but is relatively more common in those between puberty and 50 years of age. It occurs much more frequently in women than in men, a ratio of female to male patients of approximately 3:1 having been found repeatedly.<sup>421</sup>

Fig 34-2 Age and sex distribution of idiopathic thrombocytopenic purpura 66 cases in Salt Lake City<sup>19</sup> compared with 271 cases from New York,<sup>14</sup> Philadelphia<sup>33</sup> and Baltimore<sup>137</sup>





the "ITP factor" is indeed an antibody have met with limited success.<sup>26,478</sup> The *complement fixation test*, which has proved well suited to the study of drug-induced platelet autoantibodies and platelet isoantibodies, has yielded essentially negative results in ITP.<sup>115-478</sup> The presence of *platelet agglutinins* has been reported by several groups.<sup>47,49</sup> In one series, positive results were obtained in 100 out of 132 consecutive cases of ITP.<sup>49</sup> However, contradictory results have been obtained with this and related tests in different laboratories,<sup>49,371</sup> and it is probable that in many cases platelet agglutination represented an artefact, ie, platelet clumping by traces of thrombin in serum.<sup>56</sup> Experience with various direct and indirect *antiglobulin tests* has been similar; ie, initially enthusiastic reports were soon followed by inconsistent results.<sup>115,123,364,476,493</sup> Like platelet agglutination, this technique is relatively insensitive and is of questionable validity in ITP because of frequent falsely positive results.<sup>476</sup>

Preliminary results obtained with less conventional methods have been more encouraging. Measurements of platelet factor 3 "availability"<sup>197</sup> or the release of <sup>14</sup>C-5-hydroxytryptamine<sup>50a</sup> or <sup>51</sup>Cr-chromate from labeled platelets are sensitive indices of platelet injury by antibodies. With these methods ("immuno-injury" techniques), antibodies were detected in 65% of patients with ITP.<sup>61</sup> Such techniques are even more sensitive when platelets with an abnormal propensity to immunologic injury, such as those from patients with paroxysmal nocturnal hemoglobinuria, are used in the test system.<sup>2</sup> Despite the increased sensitivity of these methods, negative results were obtained in a significant number of patients with otherwise typical ITP, and positive results were obtained in those with many other conditions, eg, systemic lupus erythematosus (SLE), inflammatory disorders, lymphoreticular disorders.

Still other modifications, refinements, and immunologic systems have been described,<sup>4,44,78,231</sup> but a uniformly satisfactory serologic technique for demonstrating and quantifying the antibody that presumably is

present in ITP has yet to be described. It is probable that a blocking antibody is present in some patients with ITP<sup>475</sup> and that, in others, the platelets are injured by concentrations of antibody that are undetectable in vitro. The latter phenomenon has been clearly demonstrated in studies of well-defined isoantibodies and drug-induced autoantibodies, to be discussed below.

Various other processes may lead to platelet injury in ITP. The possible presence of cell-mediated immunologic processes was suggested by studies demonstrating the capacity of platelets from patients with chronic ITP to induce in vitro lymphocyte transformation,<sup>93</sup> and to enhance the uptake by lymphocytes of <sup>14</sup>C-labeled thymidine.<sup>140</sup> Studies demonstrating inhibition of leukocyte migration by autologous platelets in this disorder<sup>19</sup> provide additional support for this hypothesis, and further suggest the presence of a blocking antibody that may inhibit delayed hypersensitivity. These data led to the hypothesis that a fluctuating balance between cellular and humoral immunologic phenomena is the major determinant of platelet injury in ITP.<sup>19</sup>

A different immunologic phenomenon may be involved in the pathophysiology of the acute self-limited form of the disorder. The frequency with which acute ITP is associated with an antecedent viral infection and the characteristic latent period between the acute infection and the onset of thrombocytopenia have led to the suggestion that a viral antigen-antibody complex, rather than a platelet autoantibody, may be responsible for platelet sensitization and sequestration.<sup>304</sup> This mechanism is discussed further on page 1101.

### Role of the Spleen

The importance of the spleen in the pathogenesis of ITP is suggested by the therapeutic effectiveness of splenectomy in this disorder. In 1916, Kaznelson proposed that the spleen acts as a filter to remove platelets from the circulation,<sup>64</sup> a hypothesis that remains consistent with presently available data.

Despite the frequent use of terms such as "thrombocytolytic," there is little evidence that platelets are aggregated or "lysed" within the circulation in ITP.<sup>115</sup> Rather, the platelets are sensitized and then removed from the circulation. When <sup>51</sup>Cr-chromate-labeled isologous platelets are administered to patients with ITP, external scintillation counting reveals a rapid accumulation of radioactivity predominantly in the spleen.<sup>3</sup> Platelet phagocytosis by splenic leukocytes has been demonstrated *in vitro*.<sup>80</sup> The rate of splenic platelet sequestration is relatively slow in most cases, and in severe cases appeared to be limited mainly by the rate of splenic blood flow.<sup>3</sup> Preliminary evidence suggests that young platelets are preferentially sequestered in the spleen.<sup>480</sup> Hepatic sequestration of platelets has been documented in ITP, usually in patients with severe thrombocytopenia and markedly shortened platelet survival.<sup>3,45</sup> The administration of small amounts of plasma from patients with ITP to normal subjects produced mainly splenic sequestration, whereas larger amounts led to hepatic sequestration.<sup>116</sup> A similar pattern of sequestration was demonstrated when platelet antibodies were infused into animals.<sup>334</sup> These data suggest that the factors involved in the sequestration of platelets in ITP are quite similar to those that determine the sequestration of erythrocytes damaged by antibodies (Chapter 27). The value of measurements of *in vivo* platelet sequestration in predicting the outcome of splenectomy in ITP is discussed on page 1087.

Reticuloendothelial sequestration, including that which occurs in the spleen, is inhibited by corticosteroids and facilitated by estrogens.<sup>116,438</sup> An estrogen-induced increase in the rate of platelet sequestration in the spleen may explain the high incidence of ITP in women of childbearing age, and the frequency with which the disease appears or relapses at the menarche and during pregnancy.<sup>371</sup>

It has been suggested that the spleen also is important in ITP as a site of production of platelet antibodies.<sup>58,61,62</sup> An IgG globulin that acts as a platelet antibody has been ex-

tracted from spleens of patients with ITP,<sup>61</sup> and is synthesized by splenic cells in tissue culture.<sup>62</sup> Splenic tissue from patients with ITP produces more immunoglobulin than that of normal controls, and a significant percentage of that formed binds to homologous platelets.<sup>70</sup> The high levels of antibody within the spleen may favor sensitization of platelets.<sup>1</sup> Following splenectomy, the titer of platelet antibodies falls in some subjects.<sup>6</sup> Even though the spleen appears to be a site of antibody production, there is as yet little direct evidence that this is especially important in the pathophysiology of ITP.

### *Role of Impaired Thrombopoiesis*

The pathophysiologic role played by megakaryocyte damage or suppression in ITP remains unclear, despite a controversy that antedates the presently accepted immunologic theory by many years.<sup>28,39,119,491</sup> Evidence for such a process is largely indirect. Thus, the absence of platelet "budding" from megakaryocytes and the morphologic abnormalities of these cells which are commonly seen in ITP have been interpreted as evidence of a suppressive or injurious effect on the precursor cell. A different explanation for these changes seems more likely, however; ie, they are the result of accelerated platelet production in response to peripheral platelet destruction. Similar abnormalities are found in animals<sup>361</sup> and in man<sup>115</sup> rendered thrombocytopenic by thrombocytapheresis, and in various forms of thrombocytopenia caused by accelerated platelet destruction by nonhumoral processes, eg, hemangioendotheliomas, thrombotic thrombocytopenic purpura (Chapter 28), intravascular coagulation (Chapter 38).

In chronic ITP, a plasma globulin has been demonstrated on the surface of the megakaryocytes by means of immunofluorescent techniques.<sup>67,77,95</sup> This is not surprising since the platelet and the cytoplasm of its precursor are identical in most respects, including their antigenic structure.<sup>26,111,118,494</sup> It is conceivable that this antibody might impair platelet

production. Large doses of heterologous antibodies impair platelet production in animals,<sup>191</sup> and indirect evidence of impaired thrombopoiesis has been found in some patients with severe ITP.<sup>336</sup> Thrombokinetic studies suggest that in ITP, platelet production, although insufficient to balance the rapid rate of destruction, is nevertheless increased.<sup>181</sup> Thus, total megakaryocyte mass ("total" thrombopoiesis) and the platelet turnover rate ("effective" thrombopoiesis) were two to nine times normal in 16 cases.<sup>45</sup> A rough correlation between the severity of the thrombocytopenia and the rate of platelet production was demonstrated; in severely affected patients, platelet production approximated the maximal precursor response.<sup>45,390</sup>

In summary, it is probable that the antibodies present in ITP interact with the megakaryocyte, but the possibility of a minor component of deficient or ineffective thrombopoiesis cannot be excluded by presently available methods.

### Role of Vascular Injury

Various clinical observations have led to the widely accepted hypothesis that direct vascular injury is of pathogenetic importance in ITP. Thus, the severity of hemorrhage seldom correlates well with the platelet count, and bleeding often is more troublesome in ITP than in secondary thrombocytopenia of equal severity, eg, aplastic anemia. Furthermore, the administration of corticosteroids or splenectomy may restore the bleeding time to normal and diminish the severity of the bleeding manifestations before, or in the absence of, an increase in the platelet count. More direct evidence usually cited in support of this hypothesis<sup>6,34,69,122,129</sup> is unconvincing, and an alternative explanation for the findings described above is equally plausible; viz, the platelets made available by any slight increase in the balance between platelet production and destruction, although not sufficient to increase the circulating platelet count, may improve hemostasis.<sup>216</sup> Such a phenomenon may explain the cessation of bleeding that may follow platelet transfusions

in ITP even though there is no increase in the platelet count.

### Clinical Picture

In *acute ITP*, the onset of the disorder usually is sudden (Table 34-4). The frequency with which a history of antecedent infection precedes the onset of bleeding has been documented repeatedly.<sup>16,271</sup> In one series, such infections were noted within three weeks preceding the onset of ITP in 84% of the cases.<sup>75</sup> Common childhood exanthems and viral respiratory diseases are the most common; rarely, acute ITP has followed vaccination.<sup>263,299</sup> In *chronic cases*, the onset usually is insidious. A long history of mild hemorrhagic symptoms is commonly obtained from the patient, but a history of antecedent infection is uncommon.

Even though thrombocytopenia is likely to be marked, the clinical manifestations of acute ITP in children are rarely severe.<sup>43</sup> In the rare adult with the acute form of the disorder, however, hemorrhage and a fulminant course may be seen.<sup>4</sup> In chronic ITP, mild to moderate bleeding is the usual finding.

Fever of mild degree may be present, and the spleen tip is palpable in approximately 10% of the patients.<sup>57,371</sup> However, the spleen never extends more than 2 to 3 cm below the costal margin.<sup>341,371</sup>

The hemorrhagic manifestations in ITP are of the "purpuric" type (Table 33-1, page 1044), and the following description applies, with few exceptions, to thrombocytopenia of any etiology.

### Skin and Mucous Membranes

Spontaneous bleeding into the skin in the form of *petechiae* is characteristic. These lesions are minute, red hemorrhages that range in size from that of a pinpoint to that of a pinhead (Fig. 33-1, page 1045). They are flat, do not blanch on pressure (Table 36-2, page 1138), and appear and regress, often in crops, over a period of days. They are most conspicuous in areas of vascular stasis such as

the dependent portions of the body and areas subjected to constriction from girdles or stockings, and on skin surfaces over bony prominences, eg, the ankle. The presence of petechiae on the face and neck is unusual except as the result of coughing. The patient may confuse these characteristic lesions with freckles or a rash.

*Ecchymoses* may develop on any skin surface. In ITP, they are seldom associated with subcutaneous hematomas, and infrequently spread or dissect into deeper or adjacent structures. Large, purple, superficial ecchymoses may be seen, particularly on the back and thighs. Circular ecchymoses frequently surround even atraumatic venipuncture sites, but external bleeding from such sites is uncommon.

Attention has been called to the occasional association of chronic leg ulcers with ITP, but this is rare.<sup>139</sup>

*Hemorrhagic vesicles or bullae* may be seen inside the mouth and on other mucous surfaces. These lesions are particularly common in a form of acute thrombocytopenia known as *onychia*, and in acute thrombocytopenia due to drug-induced antibodies (page 1091). They probably are the result of severe acute thrombocytopenia, rather than a specific feature of any particular pathogenetic form.

*Gingival bleeding and epistaxis* are common. The latter usually responds for a time to conservative measures such as nasal packing or tamponade, often to recur again and again. Epistaxis may originate from lesions resembling petechiae in the nasal mucosa, but, in many subjects, discrete bleeding points cannot be identified. Such lesions also may be found in the mucous membranes of the throat and mouth, sometimes in the absence of cutaneous hemorrhage.

The *genitourinary tract* is a frequent site of bleeding. Menorrhagia may be the only symptom of ITP,<sup>371</sup> and may appear for the first time at puberty or even before. Rupture of the hymen may be followed by profuse hemorrhage. Hematuria also is a common symptom. The blood may come from the renal pelvis, the bladder, or the urethra. Bleeding into the kidney parenchyma is rare.

*Gastrointestinal bleeding*, manifested by melena or less frequently by hematemesis, may be an important feature of ITP.

### Central Nervous System

Intracranial hemorrhage is the most serious complication of ITP, but fortunately is rare. It occurs in 1% or less of the patients.<sup>18,75</sup> The hemorrhages usually are subarachnoid in location. They are often multiple and vary in size from petechiae to large extravasations of blood.<sup>74</sup>

Numerous small hemorrhages often are seen in the retina; subconjunctival hemorrhage is not uncommon.

### Bleeding Following Trauma

Excessive bleeding frequently follows tooth extractions, tonsillectomy, or other operations or injuries, and may first attract attention to ITP. In contrast to the hereditary coagulation disorders, such traumatic bleeding is seldom voluminous or rapid. However, in contrast with even the severe hemophiliac, slow persistent oozing may follow trivial cuts, eg, razor nicks, scratches. Delayed bleeding and spontaneous hemarthrosis, characteristic of the hereditary coagulation disorders, are extremely rare in ITP.

### Clinical Course

The clinical course of ITP is quite variable, but the differences between acute and chronic cases are striking (Table 34-4). In *acute ITP*, the disorder usually is self-limited and *spontaneous remissions* occur in as many as 93% of the patients.<sup>75</sup> The duration of the disease ranges from a few days to a few months and averages four to six weeks.<sup>84,89</sup> The favorable prognosis of ITP in children (Fig. 34-4) reflects the preponderance of the acute form in this age group. In patients with *chronic ITP*, a fluctuating clinical course usually is noted. Episodes of bleeding may last a few days or a few weeks, and may be *intermittent*<sup>4</sup> or even *cyclical*.<sup>338</sup> Cessation of

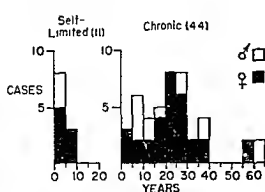


Fig. 34-4 Age and sex distribution of 55 cases of idiopathic thrombocytopenic purpura

bleeding may be associated with a rapid increase in the number of platelets, even to values above normal, but more often thrombocytopenia of variable degree persists. Spontaneous remissions are uncommon and are likely to be incomplete.<sup>53</sup> Relapses in some cases appeared to be associated with vaccinations and exposure to insecticides.<sup>209</sup> Not infrequently, the clinical course is surprisingly benign. In one patient, who refused splenectomy, chronic ITP with platelet counts averaging  $50 \times 10^9/l$  has persisted for 40 years without serious complications or treatment.<sup>130</sup>

## Laboratory Findings

### The Blood

The platelets may be totally absent or only slightly decreased in number. Bleeding is uncommon if the platelets number in excess of  $50 \times 10^9/l$ , but the platelet count often correlates poorly with the severity of the hemorrhagic manifestations, particularly in chronic cases.

Abnormalities in *platelet size and morphologic appearance* are common. The platelets often are abnormally large, eg, 3 to 4  $\mu m$  in diameter, and reveal more than normal variation in size and shape. These changes are attributable to accelerated thrombopoiesis, and are most apparent in chronic cases. The presence of such "megathrombocytes" provides indirect evidence of accelerated platelet production, and correlates roughly with the

number of megakaryocytes in the marrow.<sup>245</sup> Large platelets are commonly present for months following successful splenectomy.<sup>61</sup> More striking morphologic changes may be seen in some subjects, eg, "giant" forms 10  $\mu m$  or more in diameter, minute platelets, bizarre shapes, and deeply stained forms. Sometimes, megakaryocyte fragments may be noted in the blood smear.

Qualitative abnormalities of platelet function also have been described in ITP,<sup>11</sup> and have been attributed to coating of the platelets with antibody.<sup>20</sup> There is little evidence that these abnormalities are of significance in the production of bleeding, and some of the reported results may merely reflect the predominantly young platelet population that is usually present in this disorder.

Anemia, if present, is proportional to the extent of blood loss and is usually normocytic. If bleeding has been severe and long continued, iron-deficiency anemia develops. Occasionally, if there has been a recent severe hemorrhage, there may be reticulocytosis and moderate macrocytosis.

The *total leukocyte count* and the *differential count* usually are normal, except for those changes resulting from acute bleeding, eg, slight to moderate neutrophilia with some increase in immature forms. In some patients,<sup>23,66</sup> particularly children, eosinophilia has been noted, but this is by no means a consistent finding. Lymphocytosis with abnormal cells resembling those found in infectious mononucleosis also has been reported.<sup>85,103,128</sup>

Tests of *hemostasis and blood coagulation* reveal only those changes attributable to thrombocytopenia, ie, a prolonged bleeding time, absent or deficient clot retraction, positive reaction to the tourniquet test, and deficient prothrombin consumption. Since platelet enumeration is now far more accurate than any of these confirmatory tests, the tests are not indicated merely to confirm the presence of thrombocytopenia, as they once were. The results of tests of blood coagulation, eg, prothrombin time, partial thromboplastin time, coagulation time, are normal in patients with uncomplicated thrombocytopenia.

## The Marrow

Alterations in the bone marrow in ITP are limited to the megakaryocytes except for the normoblastic hyperplasia that may develop as the result of blood loss. The leukocytes are essentially normal. Eosinophilia has been described in many patients with acute ITP,<sup>75</sup> but the assertion that this finding is an index of good prognosis has not been confirmed.<sup>30,37</sup>

The megakaryocytes (Plate XIV) are plentiful in number,<sup>28,394</sup> and usually are increased in size.<sup>395</sup> "Young" forms with single nuclei, scanty cytoplasm, and relatively few granules are commonly seen. Morphologic abnormalities of these giant cells are present in most patients with ITP.<sup>39,91,459</sup> Vacuoles of various sizes may be present in the cytoplasm, particularly in the periphery; degenerating forms are not uncommon.<sup>459</sup> Unusually small and exceptionally large megakaryocytes with non-lobulated nuclei, many vacuoles, and few granules in the cytoplasm also have been described.

Examination of the bone marrow is helpful mainly in ruling out the possibility of other conditions with which ITP may be confused. The changes summarized above are similar to those found in most forms of thrombocytopenia caused by accelerated platelet destruction (Table 34-3),<sup>258</sup> and are not characteristic or diagnostic of ITP. The differences between megakaryocytes found in the acute and chronic forms of ITP are not clearcut,<sup>65,73</sup> and marrow examination is not particularly helpful in determining prognosis. Differential counting of the megakaryocytes has proved useful in some hands,<sup>65,459</sup> but is not widely used.

## Miscellaneous

The plasma  $\beta$ -glycerol acid phosphatase level may be elevated in any form of thrombocytopenia caused by accelerated platelet destruction.<sup>443</sup> The utility of this method in differentiating between thrombocytopenia caused by accelerated platelet destruction and that resulting from deficient production has

been questioned,<sup>358</sup> and the technique has not been widely accepted as a diagnostic adjunct in ITP.

Measurements of platelet survival and sequestration are of uncertain diagnostic or prognostic value. Like the aforementioned serologic tests, these techniques are important tools for elucidating the pathophysiology of the syndrome, but with extant methods the information obtained is of limited clinical value.

## Differential Diagnosis

Like anemia, *thrombocytopenia is a symptom*, and the diagnosis of ITP requires that the numerous disorders that may produce secondary thrombocytopenia be excluded (Table 34-2). The importance of careful inquiry regarding *drug ingestion* or exposure to *toxic substances* cannot be overemphasized, since thrombocytopenia due to drugs or toxins often is indistinguishable from ITP. The development of thrombocytopenia in an adult, in particular, should arouse suspicion of a chemical etiologic agent, since many of the drugs associated with thrombocytopenia (Table 34-5) are used more frequently by adults than by children.

Some forms of *hereditary thrombocytopenia* (page 1100) are indistinguishable from ITP on clinical grounds. This is particularly true of the autosomal dominant forms. Furthermore, a misleading family history in the absence of objective evidence of thrombocytopenia is not uncommon in ITP.<sup>51,100,121,137</sup> A discriminating family history, careful laboratory study of platelet function, and, if necessary, examination of other family members may be required to exclude the possibility of hereditary thrombocytopenia.

*Splenomegaly*, even when present,<sup>344,371</sup> is never great. Massive splenomegaly suggests an underlying disease. Lymphadenopathy, icterus, and hepatomegaly also point to a secondary form of thrombocytopenia.

An *underlying hematologic disorder* is suggested by anemia out of proportion to blood loss, and by changes in the leukocytes not attributable to hemorrhage or complicating



infection. Persistent leukopenia suggests leukemia, aplastic anemia, a disorder of the spleen, or *disseminated lupus erythematosus*.<sup>379</sup> In the last-named disorder, thrombocytopenia may be noted months or even years before other manifestations of the disease appear,<sup>371</sup> and the possibility of this condition should always be excluded by tests for nuclear-binding antibodies, and by LE preparations. Serum protein electrophoresis also is valuable, since hyperglobulinemia is common in SLE and in other disorders associated with secondary thrombocytopenia. Serum proteins and the complement level<sup>115</sup> are normal in ITP. Increased plasma levels of fibrinogen degradation products, schistocytes in the blood smear, or coagulation abnormalities suggest *diffuse intravascular coagulation* (Chapter 38) or a *microangiopathic process* (Chapter 28). Occult carcinoma, sarcoidosis, and various lymphomas may occasionally mimic ITP.

*Bone marrow aspiration* is particularly valuable in the differential diagnosis of ITP because of the large number of disorders associated with thrombocytopenia in which characteristic changes are present in the bone marrow. The initial manifestations of acute leukemia, multiple myeloma and other paraproteinemias, myelophthisic processes, and aplastic anemia may mimic ITP. Although, as emphasized above, the changes in the marrow in ITP are not diagnostic of this disorder, the absence of megakaryocytes in a normally cellular bone marrow aspirate suggests a secondary form of thrombocytopenia.

### Treatment

The course of ITP is so varied that it has been difficult to evaluate therapeutic measures. If one overlooks the possibility of spontaneous remissions, if differences in patient population, particularly age are ignored, and if one makes observations on a small number of cases for a short enough period of time, it becomes possible to make a case for many therapeutic procedures, as a review of the earlier literature concerning this syndrome

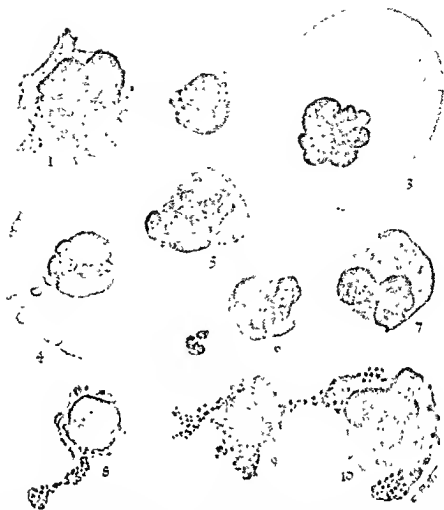
makes abundantly clear. From time to time, a large variety of remedies have found their advocates, only to be forgotten. These include, to name but a few, foreign protein injections,<sup>42</sup> turpentine, snake venoms,<sup>92,106</sup> ultraviolet light,<sup>251</sup> heliotherapy, vitamin P,<sup>70</sup> ascorbic acid,<sup>88</sup> toluidin blue, and roentgen irradiation of the spleen.<sup>82</sup> The coumarin anticoagulants and heparin were claimed to have produced beneficial effects in some cases; the rationale of such therapy is obscure. The infusion of very large doses of fresh normal plasma appeared to induce remissions in some children with acute ITP, but were ineffective in patients with chronic ITP.<sup>8,72,90</sup> The therapeutic role of all of these procedures remains uncertain. On the other hand, splenectomy and adrenal corticosteroids have withstood the test of time, and are the mainstay in the treatment of ITP. Immunosuppressive agents may be effective in patients who are refractory to these modes of therapy (page 1087).

### Conservative Management

The acute form of ITP observed in children has an excellent prognosis. As many as 93%<sup>73</sup> of affected children will make a complete recovery without any therapy. Recovery without therapy occurred within three months of onset in 75% of children with ITP and in most this occurred within four to six weeks.<sup>89,113</sup> The mortality rate is extremely low.<sup>73</sup> As a consequence, in children with mild bleeding manifestations, particularly if the disorder has developed soon after an acute infection,<sup>16,75</sup> only careful observation is indicated. Spontaneous recovery in older patients is less consistent or common, but many experienced hematologists manage mild cases in adults expectantly.

Conservative therapy is inadvisable if bleeding is severe, or if careful followup cannot be assured. It is somewhat more hazardous and unpredictable in females from puberty until the menopause, since excessive menstrual bleeding carries a risk not shared by males. The estimate of "severity" is ad-

## PLATE XIV



*Normal megakaryocyte and megakaryocytes from patients with idiopathic thrombocytopenic purpura (X1200)*

1. Mature megakaryocyte. Note granularity of cytoplasm, pseudopods, and the presence of platelets, which are situated chiefly at the periphery of the cell and in the pseudopods.

2. Megakaryoblast.

3. Mature megakaryocyte or intermediate form from a patient with chronic idiopathic thrombocytopenic purpura. There is well-defined granularity of the cytoplasm, but no platelet formation.

4. Mature megakaryocyte from a patient with chronic idiopathic thrombocytopenic purpura. There is almost complete lack of granularity and marked vacuolization and degeneration of the cytoplasm.

5. Intermediate form of megakaryocyte from a patient with acute idiopathic thrombocytopenic purpura. Granule formation, without platelet development, is evident and there are some questionable nuclear bodies (asynchronism of development) in the cytoplasm.

6, 7. Lymphoid megakaryocytes from a patient with acute idiopathic thrombocytopenic purpura. These are characterized by blue cytoplasm, lack of granularity, and lack of platelet formation.

8. Promegakaryocyte from patient with acute idiopathic thrombocytopenic purpura 24 hours after splenectomy. Nongranular platelet formation is present around the periphery of the cell, with a streamer containing a group of newly formed platelets.

9, 10. Intermediate form and mature megakaryocyte from a patient with acute idiopathic thrombocytopenic purpura 48 hours after splenectomy. There is a striking productivity of granular (functioning) platelets, seemingly with the entire cytoplasm almost ready to break up into platelets. (From Dameshek and Miller, courtesy of the authors and Grune & Stratton, Inc.)

mittedly difficult to make, and in an occasional patient the course of this disorder may become unexpectedly fulminant. However, serious hemorrhage is rare if the platelet count is  $50 \times 10^9/l$  or higher, or in patients in whom the duration of the disorder has been greater than two months.<sup>16</sup> Except for patients with very mild ITP in whom no therapy is required, expectant therapy should be limited to a period of not more than six months, since spontaneous remissions are exceedingly rare beyond this time.

### Corticosteroids

Some increase in platelet numbers and a favorable clinical response ("*partial*" remission) can be expected in 70 to 90% of patients with chronic ITP who are treated with adrenal corticosteroids. The number of patients in whom these hormones alone produce a complete normalization of the platelet count ("*complete*" remission) is much smaller, and ranges from 15 to 60%<sup>29,130,371</sup>; complete remissions of sustained duration are even less common.<sup>16,84</sup> A favorable response is usually seen in a matter of days, although, in an occasional patient, one to two months of therapy are required. The efficacy of steroid treatment is difficult to evaluate in the acute form of the disorder in children.<sup>18,75,131</sup>

**MECHANISM OF ACTION.** Corticosteroids do not increase platelet production,<sup>115,116</sup> and there is no evidence that they impair the interaction between antibodies and platelets, or that they significantly impair immunoglobulin synthesis<sup>7</sup> except perhaps after protracted administration.<sup>134</sup> Contrary to a widely held assumption, there is no direct evidence that corticosteroids reduce capillary fragility.<sup>11,101,122</sup> There is some evidence that these hormones diminish platelet sequestration in the spleen as the result of a general impairment of reticuloendothelial clearance functions.<sup>113,116,438</sup>

**INDICATIONS; REGIMENS.** Views differ regarding the exact indications for corticosteroid therapy in ITP. Although splenectomy remains the most effective measure in terms

of ultimate cure, corticosteroids are widely used as the initial therapeutic modality. It is the authors' practice to initiate corticosteroid therapy in most patients with ITP who are not selected for conservative management.

There is no evidence that any particular form of adrenal corticosteroid has any advantage over prednisone.<sup>11</sup> Various regimens have been recommended. One commonly used in initiating therapy is that of giving 40 to 60 mg of prednisone daily to adults, and 1 mg/kg body weight to children. Only in an occasional patient will larger doses produce a better response. The initial course of steroids should be maintained for three to four weeks followed by a gradual tapering of the dosage and then discontinuing the use of the drug.

If a complete remission was initially induced with steroids, but relapse occurred when their use was discontinued, there is some evidence that a second course may still produce a complete remission.<sup>130</sup> However, if the initial course of steroids produced no response, it is very unlikely that subsequent courses will be effective. When a partial remission is obtained, splenectomy, a period of expectant therapy, or maintenance at a lower dose may be indicated as dictated by individual factors.

Steroid therapy should not be maintained for long periods, a reasonable rule of thumb being six months. Although the use of modest doses of these drugs for a short time carries little risk, the often devastating effects of long-term corticosteroid therapy need little emphasis (page 555).

In patients with severe ITP who have not responded to steroids in the usual doses, it is common practice to increase the dosage to high levels (80 to 100 mg of prednisone, or even more, daily) for a brief period prior to splenectomy. In some patients, this produces some increase in platelet numbers and this may minimize surgical bleeding or serious bleeding complications. Such high doses can usually be given for three to seven days with safety<sup>134</sup>; the dosage should then be promptly tapered. The long-continued administration

of high doses (100 mg of prednisone or more) may suppress platelet formation and lead to therapeutic failure or relapse following an initial remission.<sup>24</sup> The mechanism of this effect is obscure.

### Splenectomy

The value of splenectomy in the treatment of patients with ITP has long been recognized. This procedure was first performed in 1916, at the suggestion of Kaznelson, who, at the time, was still a medical student<sup>57,63</sup>; it remains the ultimate therapeutic measure of choice in the majority of patients. Following the operation, the platelet count may increase rapidly, often within 28 to 48 hours,<sup>371</sup> and may reach levels as high as  $1000 \times 10^9/l$  or even higher in about 10 days. Not infrequently it rises more slowly. Normalization of the bleeding time and a reduction in capillary fragility may be noted before the platelet count increases.<sup>101</sup>

Complete and sustained remissions have followed splenectomy in from 50 to 88% of the patients.<sup>9 16,37 91 135</sup> In many of the remaining patients, some increase in platelet numbers and amelioration of bleeding manifestations have been observed.

**RATIONALE.** The effectiveness of splenectomy in ITP presumably is the result of the removal of the organ that is mainly responsible for the sequestration of antibody-sensitized platelets. Even when this procedure eventuates in a complete remission, evidence of increased platelet production and decreased platelet survival may persist.<sup>45 285</sup> In such cases, splenectomy appears to convert a "decompensated" state of platelet destruction into a "compensated" one, in which platelet production can keep up with continuing destruction. Evidence concerning antibody production by the spleen was discussed above. The removal of this organ may result in a reduction of antibody levels,<sup>61</sup> but this is of little apparent significance in the immediately favorable effects of splenectomy.

**INDICATIONS; CONTRAINDICATIONS.** The *indications* for splenectomy in patients with

ITP might be briefly stated as follows: (1) failure of spontaneous remission to occur after six or more months of observation in patients whose clinical manifestations are moderate or severe; (2) failure to respond to steroid therapy, occurrence of relapse following discontinuance of the use of or reduction in the dosage of steroids, and the requirement of high doses for maintenance of a clinical status free of serious hemorrhage; (3) when adequate follow-up cannot be assured, when overriding contraindications to the use of steroids are present, and, rarely, when growth, development, or social or economic status is uniquely impaired by the effects of steroid therapy or by recurrences of bleeding.

There are numerous differences of opinion regarding the exact indications for splenectomy, and, in the last analysis, the ultimate decision must be individualized.

Splenectomy is *contraindicated* in ITP: (1) early in the first episode of bleeding, especially in children, because of the frequency of spontaneous remissions; (2) in patients with acute, fulminating cases in whom the mortality following splenectomy has been high; (3) in patients with cardiac or other complications that contraindicate any major surgical procedure; (4) in children under two years of age, in whom the hazard of fulminating infection following splenectomy is greater than at a later age (page 360); and (5) in most cases of ITP in pregnant women (page 1088). Some physicians have considered acute fulminating hemorrhage to be an indication for splenectomy,<sup>46</sup> however. In such circumstances they have given platelet transfusions and steroids as well.

Other objections to splenectomy that have been raised are not well founded. It is probable that surgical morbidity and mortality due to bleeding in patients with thrombocytopenia, emphasized in the older literature, have been exaggerated. Less than 1% mortality has been the general experience in recent years.<sup>17,91,371</sup> Even patients with platelet counts as low as  $5 \times 10^9/l$  have tolerated splenectomy without undue bleeding.<sup>333</sup> The possibility that splenectomy "unmasks" or

causes an exacerbation of systemic lupus erythematosus has been cited by some,<sup>29,453</sup> but evidence for this phenomenon is almost totally lacking.<sup>84,112,339</sup> The response to the LE test may, however, become positive for the first time after operation.<sup>455</sup>

**SPLENECTOMY FAILURE.** In from 5 to 20% of patients with ITP, splenectomy produces little or no lasting benefit. Unfortunately there is no way of predicting this prior to the operation. The value of *measurements of in vivo platelet sequestration* is controversial. Of 74 patients with "predominantly" splenic sequestration in one large series, 73% enjoyed a good response following splenectomy, whereas among 19 patients with predominantly hepatic sequestration, such a response was noted in only three.<sup>87,121</sup> Others have reported less consistent results.<sup>3,4,475</sup> Measurements of the "sequestration pattern" are fraught with several methodologic difficulties,<sup>3,475</sup> and may fluctuate significantly from time to time.<sup>3</sup> "Hepatic" sequestration patterns have been documented in several patients who responded well to splenectomy.<sup>3,4,87,120</sup>

Other clinical and laboratory features of the disorder were predictive of a poor result following splenectomy in some groups of patients but not in others; eg, a high level of platelet antibodies or of "ITP factor" as measured *in vivo*, or the absence of platelet-agglutinins<sup>47</sup>; a poor response to corticosteroids<sup>130</sup>; an enlarged spleen; the absence of brisk thrombocytosis seven days following the operation.<sup>91</sup> It is probable that all of these criteria have some prognostic value, but, in practical terms, none is sufficiently reliable to exclude the likelihood of a potentially favorable response to splenectomy.<sup>3,4,58</sup> The duration of the disease prior to splenectomy<sup>344</sup> appears to be of no prognostic significance.<sup>29,55,130</sup>

Accessory spleens are mentioned frequently as a cause of failure of splenectomy. These aberrant organs are most commonly located in the splenic pedicle, the pancreas, and the peritoneum in the immediate neighborhood of the spleen, but in rare instances they have

been found in the pelvis—near the ovaries in women and in the scrotum in males.<sup>35</sup>

Radiologic techniques involving the injection of Thorotrast or red cells labeled with <sup>51</sup>Cr-chromate or mercury have been of dubious value in localization of accessory spleens.<sup>73,407</sup> This problem may be solved by newer, more sensitive scanning techniques, eg, those using <sup>99m</sup>Tc.<sup>22,32</sup>

In a few patients, surgical exploration has revealed accessory spleens and their removal has brought about remissions.<sup>35</sup> Opinions concerning the likelihood of such a successful result of exploration differ widely.<sup>16,27,421</sup> Surgeons have learned to look for accessory spleens at the time of the original operation and also to take care that implantation splenosis does not occur as the result of rupture of the spleen. In most patients with ITP in whom splenectomy fails to effect a "cure," other explanations must be sought.

The mainstay in the treatment of patients who have failed to respond to splenectomy is corticosteroids. In such patients, 40 to 60 mg of prednisone are administered until the platelet count reaches a maximum. The dose is then progressively tapered to the minimum required to control bleeding effectively. The severity of hemorrhagic manifestations, rather than the platelet count, should be the major criterion. Careful follow-up and frequent changes of dosage may be required, and sound clinical judgment regarding the occupational, financial, and psychologic effects of both bleeding and hypercorticism is essential. Some patients, observed for years with platelet count of 10 or even  $5 \times 10^9/l$  have had no significant bleeding other than some ecchymoses or petechiae, even with no steroid therapy.

Preliminary results with immunosuppressive drugs have been encouraging in some cases of ITP, but not in others.<sup>8</sup> Azathioprine,<sup>12,54,127</sup> cyclophosphamide,<sup>68</sup> actinomycin,<sup>76</sup> and other agents,<sup>12</sup> either given alone or in combination with corticosteroids,<sup>12</sup> have produced sustained remissions in some subjects. This mode of therapy has yet to be thoroughly evaluated, but the reported results are nevertheless noteworthy since they were ob-

tained in patients who had failed to respond to splenectomy or corticosteroids. Immunosuppressive therapy should probably be reserved for such refractory cases.<sup>36</sup>

### Supportive Measures

Physical activity should be restricted so as to minimize the hazards of trauma. Blood loss should be treated as otherwise indicated (page 698). Fresh blood provides more viable platelets than stored blood, but transfusion of 1 to 2 units, as may be required for the repletion of red cell mass, does not provide significant numbers of platelets. If it is desired to administer platelets, concentrates should be employed<sup>333</sup> (Chapter 12). However, such *platelet transfusions* are less effective in this disorder than in secondary forms of thrombocytopenia.<sup>507</sup> Even large numbers of platelets produce only a slight and transient increase in the platelet count, no doubt because of the rapidity with which they are sequestered *in vivo*.<sup>333, 507</sup> Platelet transfusions nevertheless may diminish bleeding for a time, and in some patients appeared to be effective in the management of serious complications, eg, subarachnoid hemorrhage. They should be reserved for such life-threatening emergencies, or for the immediate preoperative treatment of patients with serious hemorrhage prior to splenectomy.<sup>333</sup> In most patients with platelet counts above  $50 \times 10^9/l$ , preoperative platelet transfusions are not indicated.<sup>333</sup> Platelet replacement should be avoided in patients with chronic ITP because this may lead to the development of isoantibodies (Chapter 12). Anovulatory medications have proved useful when menorrhagia is a major complaint.

### Clinical Variants of ITP

Several variants of ITP have unique features worthy of mention. *Onyala* is a disorder of unknown cause observed in Africa.<sup>71, 124</sup> Hemorrhagic bullae inside the mouth and on other mucous surfaces are particularly common. With the exception of this manifestation, a possibly shorter course, and a tendency to affect young adult males as well as

children, this form of acute thrombocytopenia in the Bantu does not differ from ITP.<sup>494, 83</sup>

Several cases of ITP have been associated with the presence of *lipid-laden macrophages* in the spleen. Inclusions of at least two different types have been described, ie, those composed mainly of cholesterol<sup>64</sup> and those containing a ceroid-like substance.<sup>109</sup> This finding was first noted in patients with ITP who did not respond favorably to splenectomy<sup>31</sup> and is most common in those who have received large amounts of corticosteroids.<sup>18, 64, 110</sup> It has been suggested that the lipids may be deposited as the result of the metabolic effects of corticosteroids,<sup>64</sup> or are derived from the breakdown of sequestered platelets within macrophages.<sup>37</sup> This finding is much more common than previously suspected, and the disorder does not otherwise differ from the usual case of ITP.

### ITP in Pregnancy

The syndrome of ITP in pregnancy differs from that discussed above in four respects; namely, (1) there is a twofold increase in the incidence of spontaneous abortion<sup>102</sup>; (2) there is an increased incidence of intrapartum and postpartum bleeding as the result of thrombocytopenia; (3) thrombocytopenia and perinatal hemorrhage occur in the majority of infants borne of such mothers; and (4) corticosteroids and splenectomy may be hazardous to both mother and child.<sup>52, 377</sup>

The overall maternal mortality in pregnancy complicated by ITP is very low.<sup>81, 108</sup> However, mortality in untreated patients in whom ITP antedated pregnancy was reported to range from 7 to 11%.<sup>33, 102, 129</sup> A particularly bad prognosis has been reported in women in whom splenectomy was performed during pregnancy.<sup>129</sup> When thrombocytopenia was still present at the time of delivery, postpartum hemorrhage was slightly more common than in uncomplicated pregnancy,<sup>53</sup> and appeared to originate mainly from operative incisions and lacerations rather than from the uterus.<sup>103, 123</sup>

*Therapy* of the pregnant patient with ITP must be individualized; neither of the major

therapeutic measures is without risk. Although it has been stated that corticosteroids may induce congenital anomalies if employed in the first trimester,<sup>46</sup> this hazard appears to have been greatly exaggerated.<sup>53</sup> The therapeutic response to steroids in pregnant patients is quite similar to that which would be expected in nonpregnant patients, although there is some evidence of an increased incidence of eclampsia and postpartum psychosis.<sup>53</sup> Available data would suggest that the hazards of splenectomy exceed those of corticosteroids, and these hormones, in the doses described above, possibly supplemented with platelet transfusions at term in the severely affected patient, are preferable to splenectomy in the treatment of ITP in the pregnant patient.

Splenectomy, in common with any major surgical procedure early in pregnancy, is a well-recognized cause of abortion.<sup>102</sup> In one series, fetal mortality as high as 25% was reported,<sup>129</sup> but, in the experience of others, the incidence of fetal death was approximately the same whether splenectomy or conservative measures were employed.<sup>81, 108</sup> It is probable that the truth lies somewhere between these views, and that splenectomy constitutes a significant although not prohibitive hazard to the fetus regardless of the stage of gestation and should be avoided if at all possible.

ITP in the infant is discussed below, together with other forms of congenital immunologic thrombocytopenia.

### Pathology

The findings in the bone marrow have been described (page 1083). The spleen usually is normal in size<sup>371</sup> but may be slightly enlarged.<sup>138</sup> The average weight in one series of adult patients was 227 g.<sup>90</sup> The increase in the size of the spleen is thought to be due to congestion of the sinusoids and an increase in the size of the germinal centers of the lymphoid follicles.<sup>13, 50, 341</sup> Eosinophilic and neutrophilic leukocytes and megakaryocytes are found in the splenic sinuses.<sup>60</sup>

Histologic study of the purpuric lesions has been made.<sup>92</sup> In fatal cases of ITP, extensive hemorrhages are noted both grossly

and microscopically. Subarachnoid hemorrhage and petechial hemorrhages in virtually the entire brain ("brain purpura") are often in evidence. The spinal cord also may be involved. Subdural hemorrhage, pachymeningitis, and even hemorrhagic encephalitis also have been described.

### Other Forms of Immunologic Platelet Destruction

#### Congenital Immunologic Thrombocytopenia

Congenital immunologic thrombocytopenia in the newborn may result from the placental transfer of platelet antibodies. Such antibodies may arise from the active immunization of the mother by fetal platelet isoantigens (*isoimmune type*) (Fig. 34-5) or from the passive transfer of autoantibodies present in the maternal circulation as the result of ITP ("congenital" ITP).

### Pathophysiology

Pathophysiologically, the *isoimmune type* is similar to erythroblastosis fetalis (Chapter 27). Thus, as a consequence of the inheritance by the fetus of platelet isoantigens lacking in the mother, isoantibodies are formed in the maternal circulation; these isoantibodies cross the placenta and produce thrombocytopenia in the fetus.<sup>448</sup> Immunization to four such isoantigens has been documented, ie,  $PI^{A1}$  (ZW<sup>a</sup>),  $PL^{E2}$ ,  $PIGrLy^{B1}$ , and  $PIGrLy^{C1 475}$  (Chapter 12). Although thrombocytopenia and bleeding are infrequent, isoimmunization to platelet antigens is not uncommon; it often occurs during the first pregnancy.

Of viable infants borne of mothers with ITP, 35 to 70% will have thrombocytopenic purpura.<sup>441, 53</sup> This is due to the passive placental transfer of the "ITP factor." Such congenital ITP may develop in infants borne of mothers who are in remission following splenectomy,<sup>41, 52, 53</sup> but is uncommon when the mother has undergone a spontaneous or a steroid-induced remission.

Rarely, thrombocytopenia in the neonate results from the passive transfer of maternal

autoantibodies induced by drugs, such as quinine<sup>296,218</sup> (page 1091), and those associated with SLE.<sup>437</sup>

### Clinical Features

Irrespective of the etiologic basis, the clinical features of congenital immunologic thrombocytopenia are similar. Bleeding is seldom life-threatening, and the disorder is frequently very mild or even subclinical. Generalized petechiae and ecchymoses often are prominent, and in infants with the congenital type of ITP they are usually present at birth. In infants with thrombocytopenia of the isoimmune type, purpura often is first seen when they are one to two weeks of age. Icterus, due to indirect bilirubin absorbed from blood in the skin, is commonly present in infants with either type of the disorder.

In a minority of infants with congenital ITP, hemorrhage may be severe and the course during the perinatal period may be stormy. The first few days of life appear to be particularly critical, intracranial hemorrhage is the most common cause of death. The majority of such severely affected infants were the offspring of mothers who were thrombocytopenic at the time of delivery.<sup>41,430</sup>

### Laboratory Diagnosis

Thrombocytopenia usually is marked. In most infants with congenital ITP, the bone marrow reveals normal or increased numbers of megakaryocytes. In some cases due to isoimmunization, marked hypoplasia of the megakaryocytes has been noted and has been attributed to damage to the precursor by the antibody.<sup>475,479</sup>

Serologic tests, employing techniques such as complement fixation, which will be discussed below, will detect the presence of an antibody in approximately 70% of infants with ITP due to isoimmunization. These techniques, however, are available only in specialized laboratories. In the remaining patients the antibodies are "incomplete," and can be demonstrated only by their blocking action against isoantibodies of known speci-

ficity, or by their *in vivo* effects in normal subjects.<sup>479</sup> The serologic detection of autoantibodies in congenital ITP was discussed on page 1077.

### Differential Diagnosis

In the differential diagnosis of congenital immunologic thrombocytopenia the many and varied causes of *thrombocytopenia in the newborn* must be considered. Those unique to the neonate include various congenital infections (particularly syphilis<sup>283,321</sup>), toxoplasmosis,<sup>375</sup> cytomegalic inclusion disease,<sup>329</sup> rubella, and disseminated herpes.<sup>442</sup> Intravascular coagulation is a serious problem in the neonate<sup>442</sup>; it most commonly results from septicemia. Rarely, DIC may be "transferred" passively from the maternal circulation (page 1213). Drugs ingested by the mother, such as chlorothiazides, and congenital thyrotoxicosis<sup>503</sup> are rare causes of neonatal thrombocytopenia. Infiltration of the marrow presumably is the cause of neonatal thrombocytopenia in congenital forms of leukemia<sup>442</sup> and in the reticuloendothelioses.<sup>409</sup> Other sections in this chapter are devoted to a discussion of the hereditary thrombocytopenias (page 1100) and congenital megakaryocytic hypoplasia (page 1096).

As discussed in Chapter 9, the platelet counts in thriving *prematures* approximate those in normal neonates and adults.<sup>325,383</sup> "Physiologic" thrombocytopenia, in many cases, may well be the result of an unrecognized infection or toxin in a particular nursery.<sup>442</sup> Thus, thrombocytopenia in the premature, as in other infants or adults, requires a careful search for an etiologic factor.<sup>447</sup>

Thrombocytopenia is commonly associated with severe *erythroblastosis fetalis* (Chapter 27) but appears to be unrelated to platelet antibodies. The pathophysiology of thrombocytopenia in this disorder is complex and poorly understood. Numerous factors may be of pathophysiologic importance, including the effects of the products of red cell breakdown, bilirubin,<sup>374,489</sup> antigen-antibody complexes (page 1101), exchange transfusions,<sup>368</sup> and intravascular coagulation.<sup>352</sup>



## Treatment

In the majority of infants, no therapy is required since bleeding usually is *minimal*. However, the overall mortality due to bleeding in infants with congenital ITP ranges from nil to 25%<sup>53,102,129,330</sup>, in those with *isoimmune thrombocytopenia*, a 13% mortality rate has been reported.<sup>475</sup> The platelet count returns to normal as the responsible antibody disappears from the circulation. This usually occurs within one to four weeks, but rarely as many as four months may be required.<sup>442</sup> Recovery following *isoimmune thrombocytopenia* usually is more rapid than that following congenital ITP.<sup>479</sup>

Even though infused platelets are destroyed rapidly, platelet transfusions may be particularly effective in the infant because of the small blood volume. If immunologically reactive platelets must be administered to infants with ITP due to isosensitization, the transfusion should be given slowly. Platelets of assured compatibility can be prepared by plasmapheresis of the mother.<sup>327</sup> The therapeutic efficacy of corticosteroids and exchange transfusion<sup>53,346,405,479</sup> in these disorders remains uncertain because of insufficient data. Exchange transfusion has been recommended for infants with *isoimmune thrombocytopenia* in whom severe thrombocytopenia, jaundice, or megakaryocytic hypoplasia is present.<sup>475</sup>

### Post-transfusion Thrombocytopenia Due to Isoantibodies

The isoantibodies resulting from the transfusion of incompatible platelets are discussed in Chapter 12. The usual effect of such isoantibodies is the destruction of only the infused incompatible platelets. Rarely, however, severe thrombocytopenia is produced by the transfusion of incompatible platelets.

A unique immunologic mechanism apparently underlies platelet destruction in this condition. In all documented cases, the platelets of affected patients were negative for platelet antigen  $PI^{A1}$  ( $ZW^a$ ),<sup>475</sup> but the responsible antibody was specific for  $PI^{A1}$ .

positive platelets. A history of prior transfusion has rarely been obtained, but the disorder has been observed only in women with a history of one or more pregnancies.<sup>475,478</sup> The mechanism by which an isoantibody destroys autologous  $PI^{A1}$ -negative platelets has yet to be explained. Cross reaction with an unidentified second antibody<sup>430</sup> or antigen-antibody complexes may possibly explain the thrombocytopenia.<sup>475,478</sup>

Thrombocytopenia usually is persistent and bleeding may be severe. In one patient, the platelet count remained subnormal for two months following a single blood transfusion.<sup>430</sup> Exchange transfusion was immediately and permanently effective in two patients,<sup>354,478</sup> but no other mode of treatment is of established value. In contrast to other forms of immunologic thrombocytopenia, severe febrile reactions have followed platelet transfusions in several patients with this disorder.<sup>478</sup>

### Thrombocytopenia Due to Drug-Induced Antibodies

Thrombocytopenia due to drugs and chemical agents has become increasingly common during the past two decades. Indeed, in this age of drugs and chemicals the presence of potentially toxic agents in the environment has now become so widespread that it is seldom possible to truly exclude exogenous toxins as a cause of thrombocytopenia. It is not improbable that many cases of "idiopathic" thrombocytopenia, as well as relapses from ITP previously in remission, may be the result of occult environmental toxins.<sup>210</sup>

The numerous chemical and physical agents that may be associated with thrombocytopenia are summarized in Table 34-5. With the exception of those that suppress platelet production (group I-B) and are discussed below, it must be recognized that only a few drugs have been related to the production of thrombocytopenia beyond the statistical probability of coincidence, eg, quinine, quinidione, fudarin, Sedormid, digitoxin. In most cases, the evidence relating a given drug to the production of the dyscrasia is only

Table 34-5. Chemical Agents Which May Produce Thrombocytopenia\*

## I AGENTS THAT SUPPRESS PLATELET PRODUCTION

A Those that produce generalized bone marrow suppression (Table 56-2, page 1746)

B Those that selectively suppress the megakaryocyte

Chlorothiazides<sup>181, 411</sup>Estrogenic hormones<sup>184, 256</sup>Ethanol<sup>212, 213, 212</sup>Tolbutamide<sup>239</sup>

## II AGENTS THAT PROVOKE THE FORMATION OF PLATELET ANTIBODIES

Acetazolamide (*Diamox*)<sup>114</sup>Allyl isopropylcarbamide (*Sedormid*) andcongeners<sup>142, 141, 223</sup>Antazoline<sup>145</sup>Carbamazepine<sup>229</sup>Centulin<sup>240</sup>Chlorothiazides<sup>41, 153, 172, 181</sup>Chlorpropamide (*Diabinese*)<sup>183</sup>Desipramine<sup>234</sup>Digitoxin<sup>240</sup>Diphenylhydantoin (*Dilantin*)<sup>257</sup>Gold salts<sup>193, 252</sup>Hydroxychloroquin<sup>257</sup>Methyl DOPA<sup>152</sup>Novobiocin<sup>169</sup>Organic arsenicals<sup>275, 243</sup>p-Amino salicylic acid (PAS)<sup>187, 259</sup>Quinidine<sup>155, 159, 238, 246</sup>Quinine<sup>151, 160, 195, 209</sup>Rifampin<sup>157</sup>Stibophen (*Fuadin*)<sup>206</sup>Sulfmethazine<sup>143</sup>Sulfathiazole<sup>202</sup>

## III MISCELLANEOUS

A Those that damage circulating platelets directly

Ristocetin<sup>189, 198</sup>

B Those whose mechanism of action is unknown†‡

Acetaminophen<sup>194</sup>Allyl isopropyl barbituric acid<sup>145</sup>Aminopyrine<sup>217</sup>

Amobarbital

Aspirin<sup>228</sup>Bismuth<sup>154</sup>Butobarbital<sup>504</sup>

Carbutamide

Cephalothin<sup>184, 244</sup>Chloroquin<sup>226</sup>

Chlorpheniramine maleate

Chlorpromazine

Codeine

Colloidal silver<sup>144</sup>Copper sulfate<sup>238</sup>

Dextroamphetamine sulfate

Diazoxide<sup>143, 255</sup>

Digitalis

Digoxin

Dinitrophenol<sup>203</sup>Disulfiram (*Antabuse*)<sup>251</sup>Ergot<sup>230</sup>

Erythromycin

Ethyl allyl acetylurea (*Allymid*)<sup>145</sup>5 Ethyl phenylhydantoin (*Nirvanol*)<sup>205</sup>Heperin<sup>224</sup>Iopanoic acid (*Telopaque*)<sup>254</sup>Insecticides<sup>206, 210</sup>Isoniazid<sup>191</sup>

Meprobamate

Meproamate<sup>41</sup>Mercurial diuretics<sup>142</sup>Organic hair dyes<sup>149</sup>Nitroglycerine<sup>245</sup>Oxyphenbutazone<sup>190</sup>Oxytetracycline<sup>150</sup>Paramethadione<sup>236</sup>Penicillin<sup>200</sup>

Phenacetyl

Phenobarbital<sup>158</sup>Phenylbutazone<sup>178</sup>Potassium iodide<sup>148</sup>Prednisone<sup>24</sup>Prochlorperazine<sup>219</sup>

Promethazine

Propylthiouracil<sup>277</sup>Pyrazinamide<sup>216</sup>

Reserpine

Sodium salicylate<sup>235</sup>Spironolactone<sup>207</sup>Streptomycin<sup>227</sup>Sulfadiazine<sup>202</sup>Sulfadimethine<sup>215</sup>

Sulfamerazine

Sulfamethoxazole

Sulfamethoxypyridazine (*Kynex*)<sup>242</sup>Sulfisoxazole (*Gantisin*)<sup>186</sup>

Tetracycline

Tetraethylammonium (TEA)<sup>189</sup>Thiourea<sup>213</sup>Toluene di-isocyanate<sup>204</sup>Trimethadione<sup>178</sup>Turpentine<sup>254</sup>

\*Within groups agents are listed alphabetically

†The majority probably act by means of an immunologic mechanism, but definite evidence for antibodies is lacking

‡Agents not documented by specific case reports are cited in references 96, 143, 146, 165, 188, 196, and 201

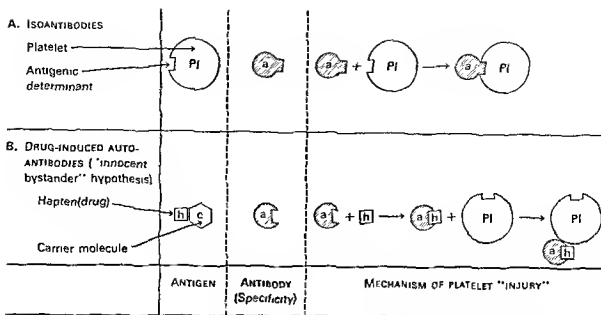


Fig 34-5 Reactions involved in immunologic platelet injury A. Isoantibodies B. Drug induced platelet autoantibodies (the innocent bystander hypothesis) Key c = carrier molecule, h = hapten (drug), pi = platelet, a = antibody

circumstantial. In the case of drugs that are taken by a significant percentage of the population, the association between the drug and the dyscrasia may be merely coincidental, eg, aspirin.

Convincing evidence for the presence of drug-induced platelet antibodies, such as positive reactions to *in vitro* serologic tests or the production of thrombocytopenia by readministration of the drug to sensitive individuals, has been documented only for those drugs listed in group II. There is indirect evidence that the drugs listed in group III-B also act by means of an immunologic mechanism. Several reviews of drug-induced thrombocytopenia may be consulted for further details.<sup>143, 146, 165, 183, 196, 201</sup>

### Pathophysiology

Drug-induced platelet antibodies are the result of an idiosyncratic reaction, which develops in only a very small proportion of persons exposed to a drug. This ranges from approximately 1 in 100,000 persons for quinidine to as many as 1 in 100 for gold salts.<sup>192</sup> The determinants of such idiosyncrasy remain obscure.

In some cases of thrombocytopenia due to hydrochlorothiazide<sup>172</sup> and in one associated with rifampin<sup>157</sup> an IgM antibody was documented. In all other cases, the responsible antibodies have been of the IgG type. There is now convincing evidence that the drug acts as a *hapten*. *In vivo*, the interaction between drug, antibody, and platelet leads to platelet injury and rapid sequestration; *in vitro*, it is manifested by various serologic phenomena, including platelet agglutination, lysis, and complement fixation. Neither the antibody nor the drug is active alone.

Numerous studies of platelet antibodies induced by quinidine, quinine, and Sedormid have provided evidence for an immunoreaction differing from that usually evident with hapten-induced antibodies (Fig. 34-5). In terms of a hypothesis proposed by Miescher and associates<sup>221, 222</sup> and by Shulman<sup>216, 474</sup> ("the innocent-bystander hypothesis"): (1) the antigen is a complex formed between the drug (hapten) and some plasma protein or other "carrier" molecule; (2) the antibody is directed against this drug-"carrier" complex, and not against any intrinsic antigenic determinant of the platelet; (3) the events leading to platelet injury involve a preliminary com-

bination between the drug and the antibody; (4) the resulting complex then attaches to the platelet by means of an essentially nonspecific process.

It is probable that degradation products of many drugs are responsible for sensitization,<sup>247</sup> a fact that may explain the absence of positive serologic reactions in many patients. In two patients with thrombocytopenia due to acetaminophen, the antibody was directed against a metabolite but not against the unaltered drug.<sup>172a,173</sup>

### Clinical Features

Thrombocytopenia of marked severity usually develops within 12 hours of ingestion of quinine, quinidine, or Sedormid by a sensitized individual. Gold salts and organic arsenicals may act more slowly.<sup>219</sup> Prodromal symptoms such as fever, chills, lethargy, and pruritus may be observed. Bleeding may be severe and usually is of abrupt onset. Hemorrhagic vesicles are commonly seen in the oral mucosa. A history may be obtained of prior ingestion of the drug followed by a latent period,<sup>223</sup> but this is not invariably the case. Exceedingly small amounts of drug may produce severe thrombocytopenia. For example, even the quinine present in tonic water may be sufficient ("cocktail" purpura).<sup>151</sup>

### Diagnosis

The megakaryocytes are normal in number, but there may be an increase in immature forms and platelet "budding" may be absent. In an occasional patient, transient hemolysis has been associated.<sup>474</sup>

Serologic verification of the presence of a platelet antibody may be made by a variety of techniques. In approximate order of sensitivity, those most commonly used are complement fixation,<sup>137,199,236</sup> "immunoinjury" techniques,<sup>61,231</sup> platelet agglutination, and inhibition of clot retraction.<sup>117,236</sup> The last named test is relatively insensitive,<sup>236</sup> but can be performed outside of an especially equipped laboratory, either during the acute phase or after the patient has recovered. Re-

cently developed methods employing platelet aggregometry (page 1054) also may prove to be suitable for the routine detection of platelet antibodies.<sup>170</sup>

The readministration of a suspected drug in an attempt to confirm an etiologic relationship is not recommended as a routine diagnostic measure.<sup>40</sup> Recurrences of severe thrombocytopenia may result unless exceedingly small amounts of drug (plasma concentrations on the order of  $10^{-6}$ ,  $10^{-7}$  M) are administered at a carefully controlled rate, such as by an automatic infusion pump.<sup>216</sup>

### Treatment

No therapy ordinarily is needed since withdrawal of the offending drug is followed by recovery. Platelet transfusions and exchange transfusion may be helpful in tiding the patient over life-threatening complications. There is little evidence that steroids are effective therapeutically, although they are widely used. When the responsible drug is quinidine, quinine, or Sedormid, thrombocytopenia usually begins to regress within 24 hours if no additional drug is taken. A normal platelet count is restored within one week, often to be followed by mild thrombocytosis. Thrombocytopenia that persists longer than two weeks is probably not due to a drug-induced platelet antibody, except in the case of drugs that are excreted very slowly. Thus, if the offending drugs are gold salts or arsenicals, protracted thrombocytopenia is the rule. BAL may accelerate excretion of these drugs.<sup>214,241,250</sup>

Although the titer of antibody may fall with time, potentially serious thrombocytopenia can be expected throughout the life of a sensitized individual, and further use of an offending drug is absolutely contraindicated.

### Miscellaneous Immunologic Thrombocytopenias

Thrombocytopenia may complicate various disorders known to be associated with disordered immunologic responses. In most

instances, it appears to result from immunologic platelet injury.

*Systemic lupus erythematosus* (SLE) may be manifested by thrombocytopenia months or even years before other manifestations of the disease appear.<sup>371,379,455</sup> Bleeding is rarely severe, and autoimmune hemolytic anemia is frequently associated. Platelet antibodies have been demonstrated in as many as 78% of these patients when the more sensitive tests have been used and are not uncommon in patients without thrombocytopenia.<sup>59,61</sup> In one series of patients, platelet survival was shortened and "megathrombocytes" were numerous,<sup>61</sup> observations suggesting the presence of compensated platelet destruction. "Amegakaryocytic" thrombocytopenia with normal platelet survival was reported in one patient with SLE.<sup>387</sup>

Other disorders in this category include certain autoimmune hemolytic anemias (Evans' syndrome),<sup>378,419</sup> chronic lymphocytic leukemia,<sup>171</sup> the lymphomas,<sup>371</sup> rheumatoid arthritis,<sup>61</sup> and hyperthyroidism.<sup>382,423,445</sup> Platelet antibodies are less commonly found in association with these disorders but may be present in the absence of thrombocytopenia.<sup>61</sup>

Thrombocytopenia may be associated with certain allergic reactions, eg, to foods,<sup>329,418,484</sup> insect bites,<sup>380</sup> tetanus toxoid,<sup>406</sup> and vaccines.<sup>294,299</sup> There is indirect evidence that platelet destruction results from an immunologic mechanism in these instances, possibly as a result of the action of antigen-antibody complexes<sup>103,487</sup> (page 1101). Diffuse intravascular coagulation (page 1211) may be an important contributory factor in the thrombocytopenia associated with heat stroke<sup>503</sup> or certain anaphylactic reactions.

### Deficient Platelet Production

Deficient platelet production may result from one of three mechanisms: (1) hypoplasia or suppression of the precursor megakaryocytes; (2) ineffective thrombopoiesis despite a normal precursor mass; or, rarely, (3) deficiency or aberration of thrombopoietic control mechanisms.

### Hypoplasia of Megakaryocytes

In uncomplicated marrow hypoplasia, platelet survival usually is normal, and thrombokinetic studies have revealed diminished "total" and "effective" thrombopoiesis.<sup>390</sup> A wide variety of exogenous factors and endogenous processes may lead to hypoplasia of the megakaryocytes and deficient platelet production. Such a process is characteristic of aplastic anemia and the Fanconi syndrome (Chapter 56). In both disorders, thrombocytopenia may be the first evidence of the disease,<sup>467</sup> and may persist after anemia and granulocytopenia have responded to treatment.<sup>470</sup>

Thrombocytopenia is uncommon in the myelophthisic processes (Chapter 57), but when present appears to result from deficient platelet production.<sup>396</sup> The mechanism by which infiltration of the bone marrow with abnormal cells impairs thrombopoiesis is unclear.<sup>272,348</sup> The number of megakaryocytes is difficult to assess accurately in most of these disorders, and quantitative studies of thrombokinetics are fragmentary.<sup>396</sup> In disseminated carcinoma, thrombocytopenia may accompany diffuse involvement of the bone marrow,<sup>499</sup> but thrombocytosis is more common. In some cases of thrombocytopenia associated with carcinoma, an immunologic process has been implicated.<sup>348</sup> As discussed elsewhere (page 1101), hypoplasia of the megakaryocytes with deficient platelet production is the major cause of the thrombocytopenia that occurs in association with certain viral infections.

In many of the above-mentioned disorders, factors other than deficient platelet production may complicate the picture.

### Chemical and Physical Agents

#### Agents That Produce Generalized Bone Marrow Suppression

Chemical and physical agents may produce thrombocytopenia as the result of a predictable suppression of the marrow, eg, ionizing radiation, alkylating agents, antimetabolites,

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cytotoxic drugs. The mechanisms by which these agents act are well defined (Chapter 55), and thrombocytopenia is a common complication when they are used in immunosuppression and in cancer chemotherapy. In addition, a larger number of drugs produce marrow hypoplasia as a result of idiosyncratic reactions, eg, chloramphenicol (Table 56-2, page 1746). The pathophysiology of thrombocytopenia in these cases is poorly understood.

Drugs of both types damage other bone marrow precursors as well as the megakaryocytes, and the usual picture is one of diffuse bone marrow hypoplasia and pancytopenia. Rarely, only thrombocytopenia may be present. The megakaryocyte is not insensitive to whole body irradiation (page 1714), and platelets frequently are the last cell type to return to normal following recovery from bone marrow hypoplasia; in some patients, thrombocytopenia may persist indefinitely.<sup>467,470</sup> Drug-induced aplastic anemia is discussed in Chapter 56.

#### *Agents That Selectively Suppress the Megakaryocyte*

*Chlorothiazide* and various of its congeners may produce thrombocytopenia by one of at least two mechanisms, ie, by the formation of platelet antibodies,<sup>142 172</sup> as already discussed, or by a poorly understood suppression of thrombopoiesis. The latter action is by far the more common. The evidence for marrow suppression rests largely on negative serologic tests for platelet antibodies and diminution in the number of megakaryocytes in the bone marrow. Mild asymptomatic thrombocytopenia may occur in as many as 25% of persons taking these agents, an observation suggesting that the thrombocytopenia may be a pharmacologic rather than an idiosyncratic effect.<sup>411</sup> In some cases, the thrombocytopenia may have been caused by severe congestive heart failure.<sup>411,445</sup> Recovery from thrombocytopenia in association with thiazide drugs is very slow, and readministration of the drug for protracted periods usually is required to reproduce thrombocytopenia.<sup>411</sup>

The administration of chlorothiazides to

pregnant women is a rare cause of congenital thrombocytopenia in the neonate.<sup>220,237</sup> The pathophysiologic mechanism apparently is suppression of the megakaryocytes. The mothers themselves are rarely, if ever, thrombocytopenic.<sup>142</sup>

*Estrogenic hormones* appear to affect platelet kinetics in animals both by facilitating reticuloendothelial sequestration<sup>438</sup> and by impairing thrombopoiesis.<sup>253</sup> Neither effect has been convincingly demonstrated in man, but several cases of "amegakaryocytic" thrombocytopenia have been reported following the administration of diethyl stilbestrol.<sup>164,256</sup> In one patient, thrombocytopenia recurred when the hormone was readministered.<sup>161</sup>

There is evidence that *ethanol* suppresses platelet production,<sup>212,213,232</sup> a phenomenon that may be a relatively common cause of mild thrombocytopenia in the alcoholic patient. In most reported cases, nutritional factors were excluded but hepatic function was impaired. Thrombokinetic studies have demonstrated accelerated platelet destruction as well as a subnormal compensatory increase in thrombopoiesis.<sup>360</sup> The megakaryocytes usually have been normal or even increased in number.<sup>212 232</sup> The experimental administration of ethanol produces thrombocytopenia in somewhat less than 50% of normal subjects.<sup>212,213 232</sup> Large doses were required and the duration of ethanol administration prior to the onset of thrombocytopenia ranged from hours<sup>232</sup> to weeks,<sup>213</sup> observations that suggest a component of individual idiosyncrasy. Bleeding is rare, and when ethanol is withdrawn the platelet count returns to normal or even supernormal levels in two to three weeks.<sup>212</sup>

*Corticosteroids* may suppress platelet formation in some patients with thrombocytopenia.<sup>24</sup> They have no clearcut effects on thrombopoiesis in normal subjects.<sup>115</sup>

#### **Congenital Megakaryocytic Hypoplasia**

The term "congenital megakaryocytic hypoplasia" includes a group of disorders having in common neonatal thrombocytopenia, marked hypoplasia of the megakaryo-

cytes, and congenital anomalies.<sup>391</sup> The similarity between the anomalies associated with this syndrome and those associated with congenital rubella suggests that the disorder is due to intrauterine fetal injury at approximately six to eight weeks of gestation.<sup>391</sup> Maternal ingestion of tolbutamide<sup>339</sup> was implicated in some cases, but with this exception no etiologic agents have yet been defined. There is evidence for a hereditary basis in some kindreds.<sup>391,468</sup>

Purpura and ecchymoses usually are apparent in these infants at birth, and serious bleeding manifestations, in particular intracranial bleeding, are not uncommon. Examination of the bone marrow reveals marked diminution in the number of megakaryocytes; in many patients they may be totally absent. A leukemoid reaction of the granulocytic type (page 1301) with myeloid hyperplasia of the marrow has been documented in as many as 50% of the patients.<sup>391</sup> These findings, as well as the platelet count, may fluctuate from time to time.<sup>391</sup>

Associated skeletal anomalies are present in virtually every subject. Bilateral agenesis of the radius is the most common.<sup>339,471</sup> In some of these infants the ulna and the humerus also are absent.<sup>370</sup> Less commonly, cardiac defects,<sup>370</sup> microcephaly,<sup>373,398</sup> micrognathia, and various other minor anomalies are seen.<sup>391</sup> Chromosome abnormalities have been demonstrated in an occasional patient, eg, trisomy D<sub>13</sub> and E 18.<sup>391</sup> Hereditary spherocytosis was associated in two cases.<sup>506</sup>

Diagnosis is rarely difficult, but the possibility of congenital rubella and some variants of the Fanconi syndrome (Chapter 56) should be excluded. Corticosteroids and splenectomy are therapeutically ineffective; treatment is mainly supportive. In a few patients, survival into adult life was associated with a gradual amelioration of the thrombocytopenia.<sup>391</sup> Early death, usually due to hemorrhage, is more common.

### Ineffective Thrombopoiesis

Thrombocytopenia is a consistent feature of *megaloblastic hematopoiesis* that results

from deficiency of vitamin B<sub>12</sub> or folic acid (Chapter 15). Although seldom severe and rarely of clinical significance, it is due to a distinctive abnormality of platelet production termed "ineffective" thrombopoiesis, which was first demonstrated by means of thrombokinetic studies (page 1103).<sup>45,396</sup> It is characterized by diminished platelet production despite the presence of an increased megakaryocyte mass, and is thus quite analogous to ineffective erythropoiesis, which is also characteristic of the megaloblastic anemias (Chapter 15).

Platelet production, whether calculated per megakaryocyte or per nuclear unit, is markedly diminished.<sup>396</sup> Although the number of megakaryocytes increases in response to thrombopoietic stimuli (Fig. 34-1), the normally concomitant increase in their volume does not occur<sup>45</sup> (Table 34-1) (Fig. 34-6). This presumably is due to impaired DNA synthesis and the consequent limitation in nuclear endoreduplication.<sup>401</sup> In stained smears, the megakaryocytes often appear hyperlobulated, and circulating platelets are abnormally large.

Moderate shortening of platelet survival time<sup>408</sup> and, rarely, hypoplasia of the megakaryocytes<sup>363</sup> may be important contributory factors in the production of thrombocytopenia in some cases.

Ineffective thrombopoiesis also has been described in the *Di Guglielmo syndrome*<sup>366</sup> and in *paroxysmal nocturnal hemoglobinuria*. In the latter disorder, a defect in the platelet membrane results in abnormal sensitivity to the action of various antibodies and of complement.<sup>2</sup> Nevertheless, platelet survival usually is normal, and the moderate thrombocytopenia commonly associated with this disorder appears to result from ineffective thrombopoiesis.<sup>396</sup> Ineffective thrombopoiesis also is present in some hereditary forms of thrombocytopenia,<sup>336,296</sup> thrombopoietin deficiency (as discussed below), and certain forms of "preleukemia."<sup>336</sup>

Moderate thrombocytopenia, which responds to the administration of iron, has been described in association with severe iron-deficiency anemia.<sup>388,420</sup> This may represent another form of ineffective thrombopoiesis, but

in some cases thrombocytopenia was more likely the result of coexistent folic acid deficiency.<sup>358</sup>

# Disorders of Thrombopoietic Control

Disorders of this type are uncommon but they are of unusual interest in that thrombocytopenia appears to result from abnormalities in the mechanisms that normally regulate platelet production.

## "Thrombopoietin" Deficiency

In one remarkable patient, thrombocytopenia appears to be the result of the deficiency of a humoral factor, possibly "thrombopoietin"<sup>326,473</sup> (page 386). The patient, now a young woman, has been studied continuously since she was discovered to have thrombocytopenia early in childhood. The transfusion of normal plasma has repeatedly induced the maturation of previously immature megakaryocytes, as well as an increase in platelet count to normal or even supernormal values. Neither corticosteroid therapy nor splenectomy has been therapeutically effective, but two spontaneous remissions of approximately one year's duration were observed. The clinical course has been complicated by one episode of hemolytic anemia of uncertain cause and another of glomerulonephritis. The patient remains responsive to plasma infusions at age 20. There is no evidence for a hereditary abnormality in this patient, but a similar syndrome has been described in several members of one kindred.<sup>495</sup>

## Unstable Thrombopoietic Regulation

There is indirect evidence that thrombopoiesis is controlled by a negative feedback mechanism which, when physiologically perturbed, may "oscillate" resulting in cyclic changes in platelet production<sup>428</sup> (page 386). Marked disturbances of this cyclic control mechanism may produce an exaggerated "oscillation," marked fluctuation in platelet production, and thrombocytopenia followed by thrombocytosis. At least two forms of thrombocytopenia as well as "rebound"

thrombocytosis (page 1107) have been attributed to this mechanism.

*Tidal platelet dysgenesis* is a rare condition characterized by thrombocytopenia and megakaryocytic hypoplasia that alternates with thrombocytosis and megakaryocytic hyperplasia at fixed intervals, usually 20 to 30 days.<sup>338,376</sup> Platelet survival is normal throughout this cycle,<sup>136,338</sup> even though thrombocytopenia may be severe in the "negative" phase. No therapy has proved effective.<sup>136,376</sup>

*Cyclic thrombocytopenia* is a more common disorder in which thrombocytopenia and thrombocytosis alternate at regular intervals, but are not accompanied by megakaryocytic hypoplasia in the "negative" phase. The disorder is most common in women, in whom thrombocytopenia develops during the menstrual period. Endocrine factors do not appear to be responsible.<sup>483</sup> Bleeding is seldom significant and there is no effective therapy.

A cyclic though irregular variation in the platelet count has been observed in thrombocytopenia caused by accelerated platelet destruction,<sup>315</sup> eg, ITP, and in children with cyanotic congenital heart disease.<sup>386</sup>

## Thrombocytopenia Due to Abnormal Platelet Pooling

### Disorders of the Spleen

The splenic platelet pool normally contains approximately one third of the total platelet mass (Chapter 9) (Fig. 34-1) and may increase as the result of a large variety of disorders having in common splenomegaly,<sup>323,339,400a</sup> eg, cirrhosis with portal hypertension,<sup>488</sup> sarcoidosis,<sup>369,451</sup> Gaucher's disease,<sup>342,425</sup> Hodgkin's disease and other lymphomas,<sup>351</sup> Felty's syndrome.<sup>392</sup> This may result in thrombocytopenia in the circulating blood despite a normal or even increased total platelet pool<sup>396,412</sup> (Table 34-1).

The mechanism of platelet sequestration in disorders associated with splenomegaly is poorly understood. Large numbers of platelets are found in spleens removed surgically, but histologic studies have not clarified the hemodynamic basis for platelet sequestration.



In contrast to the sequestration of *damaged* platelets in disorders such as ITP, the sequestration in these conditions is not an irreversible process; epinephrine administration mobilizes approximately the same proportion of the splenic platelet pool whether the spleen is normal in size or enlarged.<sup>3,45,418</sup> In portal hypertension, the platelet count is not correlated with the portal venous pressure, nor does the thrombocytopenia invariably respond to successful shunting procedures.<sup>488</sup> The role of hypervolemia in the production of thrombocytopenia in these disorders has not been well studied.<sup>359</sup>

There is evidence for accelerated platelet destruction in most instances of thrombocytopenia associated with disorders of the spleen.<sup>396</sup> The initial recovery of isotopically labeled platelets in the circulation of such patients is markedly reduced.<sup>331,332</sup> Platelet survival usually is moderately shortened<sup>396,408</sup> although it may be normal.<sup>356</sup> Thus platelet pooling, even if an essentially passive process, is not entirely innocuous.

The clinical picture in "hypersplenic" thrombocytopenia usually is dominated by the underlying disease, and numerous other abnormalities of hemostasis and coagulation may be present, eg, in cirrhosis (Table 38-2, page 1207). The usual picture is one of pancytopenia, with relatively mild thrombocytopenia, and normal or moderately increased numbers of megakaryocytes in the bone marrow. In general, the severity of the thrombocytopenia correlates poorly with the size of the spleen, but it is nonetheless difficult to entertain the diagnosis of thrombocytopenia due to abnormal splenic platelet pooling in the absence of significant splenic enlargement.<sup>412</sup>

Therapy is seldom indicated for thrombocytopenia alone, but splenectomy sometimes alleviates the pancytopenia completely.<sup>450,433</sup> (Chapter 45).

### Hypothermic Anesthesia

Platelets become more "sticky," swell, and undergo various morphologic changes when stored *in vitro* at temperatures below 37°C.

This usually is a reversible process, but clumping, release of ADP, and irreversible aggregation follow protracted chilling.<sup>349</sup> Similar changes *in vivo* may underlie the thrombocytopenia that is associated with hypothermic anesthesia.

In man, mild, reversible thrombocytopenia is a predictable consequence of hypothermia below 25°C, and is usually of no clinical consequence.<sup>496</sup> In an occasional patient, thrombocytopenia persists following rewarming and may produce hemorrhage.<sup>341</sup> In some children subjected to deep hypothermia, brain damage was attributed to *in vivo* platelet clumping.<sup>341</sup> Heparin administration appears to minimize the fall in the platelet count,<sup>498</sup> but its therapeutic value is uncertain.<sup>341</sup> Many other factors must be considered in such cases, eg, extracorporeal circulatory devices, massive transfusion, intravascular coagulation.

## Miscellaneous Forms of Thrombocytopenia

### Nonimmunologic Platelet Destruction

Thrombocytopenia commonly is the result of accelerated utilization or destruction of platelets by various nonimmunologic processes. Disorders in this category (Table 34-3) are discussed elsewhere, ie, diffuse intravascular coagulation including the Kasabach-Merritt syndrome (giant hemangioendotheliomas) (Chapter 38); fibrinogenolysis (Chapter 38); thrombotic thrombocytopenic purpura and various other microangiopathic processes<sup>483a,497a</sup> (Chapter 28). Thrombocytopenia due to platelet damage by infectious agents is discussed elsewhere in this chapter (page 1101).

*Ristocetin*, a drug no longer used therapeutically, produces thrombocytopenia by a unique direct toxic action on the platelets.<sup>180,198</sup> It may also interact with the "anti-bleeding" factor that is lacking in von Willebrand's disease (page 1179).<sup>198</sup> Data suggesting that intravenous heparin administration produces thrombocytopenia by a similar mechanism<sup>182</sup> have not been confirmed.<sup>167,233,462</sup>

Thrombocytopenia, with platelet counts in the range of 50 to  $100 \times 10^9/l$ , is a predictable sequel of surgical procedures employing various extracorporeal circulatory devices.<sup>463,466</sup> It results from platelet injury during passage through the pump, and with modern equipment is no longer a major problem. Thrombocytopenia in such patients can be treated by the administration of fresh blood or platelet concentrates, but is rarely associated with significant bleeding. The presence of severe thrombocytopenia and bleeding following extracorporeal circulation suggests a complicating factor.

### Hereditary Thrombocytopenias

Hereditary forms of thrombocytopenia with diverse genetic, clinical, and laboratory features have now been documented in numerous kindreds (Table 34-6). For convenience, those forms in which abnormalities of platelet function appear to be the major feature are discussed in Chapter 35, eg, thrombopathic thrombocytopenia, the Bernard-Soulier syndrome. The Wiskott-Aldrich syndrome is discussed in Chapter 44.

Thrombocytopenia is a consistent feature of many hereditary disorders that are better known for associated abnormalities. Those involving the hematopoietic system are discussed in detail elsewhere, eg, the May-Hegglin anomaly (Chapter 42),<sup>395</sup> the Chediak-Steinbrink-Higashi anomaly (Chapter 42), hemoglobin Köln (Chapter 24),<sup>432</sup> and the Fanconi syndrome and variants thereof (Chapter 56).<sup>436</sup> In one kindred, thrombocytopenia inherited as an X-linked recessive trait was associated with increased levels of IgA immunoglobulins and renal disease.<sup>390</sup> Hereditary thrombocytopenia associated with various aminoacidurias and other inborn errors of metabolism has only recently been recognized and is of unusual interest (Table 34-6). Virtually nothing is known concerning the cause of thrombocytopenia in these conditions, and there is no known treatment. It is noteworthy that some aminoacidurias may be associated with a thromboembolic diathesis, eg, homocystinuria.<sup>372</sup>

Table 34-6. The Hereditary Thrombocytopenias

<b>I X-LINKED RECESSIVE INHERITANCE</b>	
A	Wiskott Aldrich syndrome and variants thereof (Chapter 44)
B	"Isolated" thrombocytopenia <sup>353,464</sup>
C	Thrombocytopenia with increased IgA immunoglobulins and renal disease <sup>390</sup>
<b>II AUTOSOMAL DOMINANT INHERITANCE</b>	
A	"Thrombopathic" thrombocytopenia
B	"Isolated" thrombocytopenia <sup>267,340,465</sup>
C	Varieties in which thrombocytopenia is a feature of a generalized disorder (May-Hegglin anomaly, <sup>337,365</sup> hemoglobin Köln <sup>399,452</sup> )
<b>III AUTOSOMAL RECESSIVE INHERITANCE</b>	
A	Dystrophic thrombocyteaire hemorrhagipare (Bernard-Soulier syndrome)
B	Varieties in which thrombocytopenia is a feature of a generalized disorder
1	Chediak-Steinbrink-Higashi anomaly (page 1323)
2	Fanconi syndrome and variants thereof (page 1767)
3	Inborn errors of metabolism (cystathioninuria <sup>427</sup> hyperglycinemia <sup>431,440</sup> methylmalonic and isovaleric acidemia <sup>431</sup> Sidbury syndrome <sup>422</sup> Schwachman syndrome <sup>481</sup> )
<b>IV UNCLASSIFIED FORMS</b>	
A	In association with platelet antibodies <sup>343,397</sup>
B	In association with abnormalities of platelet function <sup>454,461,469,473,485</sup>
C	In association with other heritable hemostatic defects (von Willebrand's disease, <sup>439</sup> factor IX deficiency <sup>397</sup> )
D	Miscellaneous <sup>335</sup>

Hereditary thrombocytopenia not associated with ancillary clinical or laboratory manifestations has been well documented ("isolated" hereditary thrombocytopenia). This may be inherited either as an X-linked recessive trait<sup>353,464</sup> or as an autosomal dominant trait. The latter form appears to be the more common.<sup>340,433,436</sup> The clinical laboratory features are essentially the same in both forms. Bleeding is mild and in some instances the affected family members are virtually asymptomatic.<sup>340,391,502</sup> Platelet function appears to be normal.<sup>436</sup> Megakaryocytes are normal in number, but quantitative studies of thrombopoiesis have not been reported. In one kindred,<sup>433</sup> shortened platelet survival due to an intracorporeal platelet abnormality was demonstrated.

Several other reports of hereditary thrombocytopenia do not provide sufficient data for definite classification.<sup>335</sup> In some patients the presence of platelet antibodies was described<sup>397</sup>; in others, poorly documented abnormalities of platelet function,<sup>456,461,469,472,482</sup> or additional inherited abnormalities of hemostasis or blood coagulation<sup>397,439,454</sup> were associated.

### Thrombocytopenia in Association with Infections

Purpura was recognized as a manifestation of pestilential fevers 2000 years ago. It is now known that bleeding in association with infections may be caused by several factors, among which thrombocytopenia is the most common (Table 34-2).

#### Viruses

Viral infections may produce thrombocytopenia by at least three mechanisms: (1) impaired platelet production as a result of invasion of the megakaryocytes by the virus; destruction of circulating platelets by (2) the virus, or (3) viral antigen-antibody complexes.

The administration of *live measles vaccine* produces significant though subclinical thrombocytopenia in the majority of normal children.<sup>306</sup> Degenerating, vacuolated megakaryocytes were seen three days after administration of the vaccine, at which time plasma levels of acid phosphatase were subnormal. The nadir of the platelet count was reached seven days after vaccination. These data, and studies of other viruses in animals, are consistent with the hypothesis that deficient platelet production due to parasitization of the precursor cell is a major factor in the production of thrombocytopenia. This hypothesis gains further support from *in vitro* studies demonstrating rapid viral replication within megakaryocytes.<sup>272,293,362</sup> Morphologic abnormalities of the megakaryocytes also have been described in Thai hemorrhagic fever.<sup>27,313</sup>

In other viral infections, there is indirect evidence that platelet damage results from interaction between the virus and the platelets

in the circulating blood, although damage to megakaryocytes also may be significant. Thus, in influenza and rubella,<sup>262</sup> mild thrombocytopenia has been documented during the acute febrile phase of the disease. In *in vitro* studies have demonstrated adsorption or "phagocytosis" of viruses by human platelets.<sup>316,318</sup> Such viruses produce platelet aggregation and the release reaction *in vitro*<sup>292</sup> and cause marked thrombocytopenia with reduced platelet survival in experimental animals.<sup>318</sup>

Although mild thrombocytopenia is a predictable effect within one week following the administration of live measles vaccine, in some subjects thrombocytopenia of greater severity develops two to four weeks later,<sup>293,296</sup> at a time when neither megakaryocyte damage nor acute viremia is a likely explanation. A similar phenomenon occurs in the spontaneous disease, and in rubella.<sup>304</sup> In the latter disorder, the titer of antibodies capable of inducing platelet aggregation in the presence of "small-sized" rubella antigen was much higher in children who developed thrombocytopenia following spontaneous rubella infection than in similar patients without thrombocytopenia.<sup>304</sup> This has led to the hypothesis that a viral antigen-antibody complex is responsible for platelet sensitization and sequestration.<sup>304</sup> Antigen-antibody complexes are known to induce platelet aggregation<sup>303</sup> and the release reaction *in vitro*, as well as thrombocytopenia when administered to experimental animals.<sup>302,318,432</sup> This mechanism may also explain many instances of "acute ITP" which develop following recovery from viral infections (page 1079).

Of newborns with congenital rubella, 40 to 80% are thrombocytopenic.<sup>267,276,309,314</sup> In addition to the other stigmas of this syndrome,<sup>422</sup> hepatomegaly and splenomegaly usually are present. Rarely, thrombocytopenia is the only manifestation.<sup>267,442</sup> Megakaryocytes frequently are decreased in number, and rarely may be totally absent, an observation that suggests deficient platelet production.<sup>269</sup> Schistocytes and other evidence of DIC are present in many of these infants.<sup>267,311,324</sup>

In *infectious mononucleosis* (IM),<sup>263,273</sup> subclinical thrombocytopenia is not uncommon. In the rare patient in whom severe thrombocytopenia and bleeding develop,<sup>310</sup> the diagnosis of acute leukemia may be mistakenly made. In some patients with IM, antibodies directed against a specific platelet antigen (small "i") have been demonstrated. They appear to act as platelet cold agglutinins both in vitro and in vivo (Chapter 43).

### Bacteria

Thrombocytopenia that develops in association with most severe bacterial infections is the result of DIC (Chapter 38), but other factors also may play a role. Platelet utilization on damaged vascular surfaces may be important in some cases, eg, meningococcemia.<sup>313</sup> Certain bacterial toxins, including exotoxins<sup>270,294</sup> and endotoxins,<sup>278,286a,289,293a,298</sup> produce thrombocytopenia in experimental animals, but the role of such toxins in man is uncertain.<sup>435</sup> (Chapter 38). Direct interaction between bacteria and platelets in the circulation has been demonstrated experimentally.<sup>353</sup>

### Post-transfusion Thrombocytopenia

Although brisk external hemorrhage in the absence of blood replacement usually produces thrombocytosis (page 1107), moderate thrombocytopenia commonly follows massive blood transfusions in such cases<sup>400</sup> (Chapter 11). Post-transfusion thrombocytopenia of this type is a predictable result of the transfusion of large amounts of stored whole blood, and should not be confused with that due to platelet isoantibodies (page 1091). Various factors have been implicated in the pathogenesis of this phenomenon, including the presence of a platelet clumping factor in the plasma of stored blood and platelet utilization due to embolic occlusion of the microvasculature by platelet clumps.<sup>402,424</sup> The observation that the magnitude of thrombocytopenia varies in direct proportion to the volume of blood administered<sup>410</sup> favors the view that post-transfusion thrombocytopenia

is mainly the result of the external loss of viable platelets and their replacement with nonviable platelets in stored blood. Following this "washout" phenomenon, the platelet count returns to normal slowly, ie, within three to five days, and the phenomenon is thus entirely similar to the effects of experimental thrombocytapheresis or plasmapheresis.<sup>115,361</sup> A similar process presumably explains the mild thrombocytopenia that may follow exchange transfusion, eg, in erythroblastosis fetalis.<sup>366,367</sup>

Post-transfusion thrombocytopenia seldom produces significant bleeding but can be prevented by the use of 1 unit of fresh blood for every 5 units of stored blood, and can be treated by platelet transfusions. The sequelae of occlusion of the microvasculature by platelet emboli from stored blood are probably of greater clinical significance than is bleeding.<sup>308,476</sup> The numerous other factors that may produce thrombocytopenia in association with massive hemorrhage should also be kept in mind, particularly DIC.

### Other Forms of Thrombocytopenia

Thrombocytopenia is common in cirrhosis, but is relatively rare in other types of liver disease.<sup>381</sup> Isolated thrombocytopenia in the absence of coagulation abnormalities is not uncommon in inactive cirrhosis.<sup>350</sup> The major pathophysiologic factor may be splenic platelet pooling due to portal hypertension, but, as discussed above, this remains poorly understood.<sup>488</sup>

It is generally agreed that the major cause of bleeding in *uremia* is a qualitative abnormality in platelet function (page 1129), but thrombocytopenia has been documented in as many as 50% of patients<sup>351,417,451</sup> and in some this appeared to be the major cause of bleeding.<sup>437</sup> There is surprisingly little information concerning the pathophysiology of this manifestation, and the effects of uremia on thrombopoiesis have not been defined. In some disorders associated with uremia, DIC may be present (Chapter 38).

Reports of thrombocytopenia in association with several disorders not discussed above are listed in Table 34-2. For further

details concerning these uncommon causes of thrombocytopenia, the reader is referred to the original papers.

## Thrombocytosis

Thrombocytosis refers to the presence of an abnormally high number of platelets in the circulating blood; it may result from the various pathologic processes and physiologic stimuli summarized in Table 34-7. It is a common feature of the various "myeloproliferative" syndromes such as polycythemia vera (Chapter 30), chronic myelocytic leukemia (Chapter 48), and myelofibrosis (Chapter 57). In these disorders, increased platelet numbers may be a significant pathophysiologic feature in the production of hemorrhage, thrombosis, or both. An elevated platelet count also is commonly associated with various infectious, inflammatory, and neoplastic disorders. In these conditions, the increased platelet numbers seldom produce symptoms, but often are of considerable diagnostic significance. The unqualified term "thrombocytosis" frequently is used with reference to these secondary or reactive forms.<sup>559</sup>

### Pathophysiology

Transitory thrombocytosis may result from the mobilization of extravascular platelet pools, eg, following epinephrine administration<sup>511</sup> or vigorous exercise.<sup>532</sup> Such "physiologic thrombocytosis" is discussed on page 388. All other forms of thrombocytosis apparently are the result of accelerated platelet production. Preliminary thrombokinetetic data would suggest that this may result from two different mechanisms (Table 34-8). In patients with reactive or secondary thrombocytosis, as in the normal person, the platelet count is directly correlated with the megakaryocyte mass and is inversely correlated with the mean megakaryocyte volume (Fig. 34-6). This is the result of regulatory processes that normally reduce the stimulus to nuclear endoreduplication as the platelet count rises (Chapter 9).<sup>395,396</sup> The factors leading to accelerated platelet production in

**Table 34-7. Causes of Thrombocytosis**

- I **PHYSIOLOGIC** (exercise, parturition, epinephrine)
- II **DISORDERS OF THE HEMATOPOIETIC SYSTEM**
  - A "Myeloproliferative syndromes" (thrombocythemia, polycythemia vera, chronic myelocytic leukemia, myelofibrosis)
  - B Rapid blood regeneration (following hemorrhage in various hemolytic anemias)
  - C "Rebound" thrombocytosis (following recovery from thrombocytopenia or marrow suppression)
  - D Miscellaneous (iron-deficiency anemia, hemophilia, tidal platelet dysgenesis)
- III **ASPLENIC STATES** (following splenectomy; splenic agenesis and atrophy, splenic vein thrombosis)
- IV **INFECTIOUS AND INFLAMMATORY DISEASES** (many acute infections, many chronic infections, particularly osteomyelitis, tuberculosis, ulcerative colitis, regional enteritis, rheumatoid arthritis, acute rheumatic fever, sarcoidosis, cirrhosis of the liver, Wegener's granulomatosis)
- V **NEOPLASMS** (various carcinomas, Hodgkin's disease and other lymphoreticular disorders)
- VI **MISCELLANEOUS** (following trauma and surgical procedures, osteoporosis, nephrotic syndrome and other forms of chronic renal disease, renal cysts, Cushing's disease, glycogen storage disease)

the various disorders associated with reactive thrombocytosis are almost totally obscure.

In *autonomous thrombocytosis*, platelet production apparently is unresponsive to normal regulatory processes, the platelet count does not correlate with the megakaryocyte volume (Table 34-8, Fig. 34-6).<sup>395,396</sup> and the decrease in megakaryocyte size normally resulting from an increase in the circulating platelet mass is not seen. Autonomous thrombocytosis has been likened to neoplastic proliferation of other hematopoietic elements,<sup>542</sup> and has been demonstrated in thrombocythemia and in polycythemia vera.<sup>554a</sup> Preliminary studies have led to the somewhat surprising conclusion that accelerated platelet production in chronic myelocytic leukemia is not autonomous.<sup>396,563</sup> (Chapter 48).

### Thrombocythemia

Thrombocythemia (primary, essential, hemorrhagic, or idiopathic thrombocythemia)

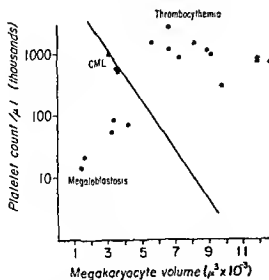


Fig. 34-6 Thrombokinetics in autonomous thrombocytosis. The expected relationship between megakaryocyte volume and the circulating platelet count as shown by the solid line is not seen in two clinical settings. In megaloblastosis the limitation of nuclear replication appears to restrict endomitosis, and hence megakaryocyte volume, despite the thrombocytopenia. In contrast megakaryocyte volume is increased in thrombocythemia (primary thrombocytosis) due to the autonomous endoproliferation. (From Harker,<sup>395</sup> courtesy of the author, the International Academy of Pathology and William & Wilkins Company.)

is characterized by abnormal proliferation of the megakaryocytes. It is manifested clinically by bleeding and a thromboembolic diathesis.<sup>530,542-571</sup> The disorder is rare, is

most frequently seen in persons in middle adult life, and affects men and women with equal frequency.<sup>542</sup> Thrombocythemia belongs in the spectrum of "myeloproliferative" disorders,<sup>560</sup> a concept that is discussed elsewhere (Chapter 46).

### Pathophysiology

Thrombocythemia is the prototype of "autonomous" thrombocytosis, discussed above. Marked increases in megakaryocyte number, total megakaryocyte mass, and mean megakaryocyte volume are characteristic<sup>395,396</sup>; platelet production may be increased to as much as 15 times normal.<sup>396</sup> The life span of platelets usually is normal<sup>395,396,565,565</sup>, reports of prolonged platelet survival<sup>509</sup> have not been confirmed.<sup>396,511,570</sup> Shortened platelet survival has been demonstrated in a few cases,<sup>334</sup> and may be the result of platelet destruction in the spleen (page 1098).

The mechanisms by which marked expansion of the circulating platelet mass produces either hemorrhage or thrombosis have not been defined.<sup>533</sup> There is preliminary evidence that the major factor in the production of hemorrhage is an intrinsic qualitative abnormality of platelet function, possibly an abnormality of the release reaction. The re-

Table 34-8. Differentiation of Thrombocythemia and Reactive Thrombocytosis

	Reactive Thrombocytosis	Thrombocythemia
<b>THROMBOKINETIC FEATURES</b>		
Total megakaryocyte mass	Slightly increased	Greatly increased
Megakaryocyte number	Increased	Increased
Megakaryocyte volume	Decreased	Increased
Platelet turnover or production rate	Increased	Increased
Total platelet mass	Increased	Increased
Platelet survival	Normal	Normal to slightly decreased
<b>CLINICAL AND LABORATORY FEATURES</b>		
Thromboembolism and hemorrhage	Uncommon	Common
Duration	Often transitory	Usually persistent
Splenomegaly	Absent*	Present in 80% of cases
Platelet count	Usually $< 1,000 \times 10^9/l$	Usually $> 1,000 \times 10^9/l$
Bleeding time	Usually normal	Often prolonged
Platelet morphology and function	Usually normal	Often abnormal
Leukocyte count	Usually normal*	Increased in 90% of cases

\*Unless as the result of the underlying disorder  
Thrombokinetetic data from Harker.<sup>395,396,545</sup>

ported findings have varied greatly from patient to patient, however, and are discussed in Chapter 35.<sup>557,566</sup> Most evidence would suggest that *thrombosis* is the consequence of the greatly increased platelet mass, together with a qualitative abnormality of the platelets.<sup>559,591</sup> The role of platelets in the pathophysiology of thrombosis is discussed in Chapter 39.

### Clinical Picture

Recurrent gastrointestinal hemorrhage<sup>580</sup> and epistaxis are the most common bleeding manifestations,<sup>516,542</sup> although hazardous hemorrhage may follow trauma or surgical operations, including even trivial biopsy procedures. Easy bruising is not uncommon, but petechiae are rarely seen.

Thrombosis of both veins and arteries may develop, and vessels in unusual sites are frequently involved, eg, the hepatic veins,<sup>539</sup> mesenteric vessels, axillary artery,<sup>539</sup> the veins of the penis with resulting priapism,<sup>542</sup> the digital vessels with resulting ischemic lesions of the toes.<sup>521</sup> Occlusion of the splenic vein<sup>542</sup> appears to be particularly common. Pulmonary embolism may follow thrombosis in any location, and is a frequent complication.

Significant splenomegaly is found in approximately 80% of these patients.<sup>542</sup> Splenic atrophy and infarction have been documented in several subjects, however.<sup>513,561,583</sup> Hepatomegaly of moderate degree is not uncommon, but lymphadenopathy is rare.

Peptic ulcer disease<sup>574</sup> and varices of the gastric and esophageal veins are not uncommonly associated with thrombocythemia<sup>513,543</sup>; rarely the signs and symptoms of secondary gout are seen.<sup>526</sup>

The clinical course of thrombocythemia is quite variable, and episodes of bleeding or thrombosis often are followed by long periods during which the patient is free of symptoms. In some cases, a benign course over a period of years has been documented despite very high platelet counts.<sup>536</sup> Thromboembolic complications are the usual cause of death.<sup>583</sup>

### Laboratory Findings

In the majority of patients with thrombocythemia, the platelet count exceeds  $1,000 \times 10^9/l$ , and counts as high as  $14,000 \times 10^9/l$  have been recorded.<sup>536,542</sup> As a consequence, platelet enumeration is often inaccurate. The determination of the volume of packed platelets (page 373) provides a useful semiquantitative method of measuring platelet numbers in this disorder. In stained blood smears, the platelets usually are clumped into large masses. Abnormalities of size, shape, and structure often are striking, eg, heavy granulation, "giant" forms, bizarre abnormalities of shape. Microcytosis of the platelets also has been described.<sup>520</sup> Megakaryocyte fragments may be present in the blood.

Morphologically the *erythrocytes* are usually normal.<sup>542</sup> Anemia, if present, usually is of the hypochromic, microcytic type,<sup>542</sup> and presumably is the result of chronic blood loss and iron deficiency. Slight erythrocytosis is present in approximately 30% of the patients.<sup>542</sup> Thrombocythemia was associated with pure red cell aplasia in one patient.<sup>517</sup> Howell-Jolly bodies and target cells may be seen in the blood smear in subjects with splenic atrophy.<sup>561</sup>

The *leukocytes* are increased in number, the total count usually ranging from 15.0 to  $40.0 \times 10^9/l$ . The differential count reveals neutrophilia and a "shift to the left," with band and juvenile metamyelocytes predominating. Occasionally myelocytes may be seen, but more immature forms are uncommon. Slight eosinophilia and basophilia are frequently present.

The *bone marrow* reveals marked hyperplasia of the megakaryocytes, which often appear in clumps or sheets.<sup>560</sup> Structural abnormalities of these giant cells and immature forms have been noted in some instances.<sup>520</sup> Hyperplasia of the granulocyte or erythrocyte precursors is not uncommon. In a few cases, myeloid metaplasia of the spleen, liver, lymph nodes, and kidneys and various minor chromosomal abnormalities have been described.<sup>539,587</sup> No characteristic histologic

picture has been noted in the spleen. Levels of leukocyte and platelet<sup>510</sup> alkaline phosphatase and serum levels of uric acid<sup>539</sup> and vitamin B<sub>12</sub> usually are elevated.<sup>526</sup>

The presence of massive numbers of platelets produces *pseudohyperkalemia*<sup>546</sup> and spurious increases in the serum levels of several other substances. These include acid phosphatase,<sup>529-530</sup> zinc,<sup>537</sup> lactic acid dehydrogenase,<sup>522</sup> acid mucopolysaccharides,<sup>552</sup> inorganic phosphorus,<sup>537</sup> and uric acid.<sup>539,546</sup> Marked thrombocytosis may lead to falsely high erythrocyte counts and grossly erroneous red cell indices when some automated counting devices are used.

Screening tests of blood coagulation usually reveal no abnormalities, but laboratory evidence of "hypercoagulability" (page 1237) is not uncommon. Signs suggesting low-grade intravascular coagulation also may be present.<sup>556</sup> The bleeding time may be prolonged or normal.<sup>562</sup> Abnormalities evidenced by tests of specific platelet functions are discussed on page 1130.

## Differential Diagnosis

Because of the magnitude and duration of the thrombocytosis and the usual ancillary findings, the differentiation of thrombocythemia from various other forms of thrombocytosis is rarely difficult (Table 34-8). In thrombocythemia, the platelet count usually exceeds  $1,000 \times 10^9/l$ , a finding that is relatively uncommon in the other forms. In many forms of reactive thrombocytosis, the elevation in the platelet count is transitory or persists for a matter of days or weeks only. Thrombocytosis persisting for months or years, unless treatment is given, is characteristic of thrombocythemia. Splenomegaly, hemorrhage, thrombosis, or morphologic or functional abnormalities of platelets are uncommon in the secondary forms unless they are the result of the underlying disease. In an occasional patient with cirrhosis of the liver, the presence of splenomegaly, varices, gastrointestinal bleeding, and thrombocytosis may lead to confusion with thrombocythemia.

Differentiation between thrombocythemia and polycythemia vera may be difficult, and in many cases this distinction appears to be largely semantic. Marked elevations of the hematocrit value in association with the signs<sup>543</sup> and symptoms of expanded blood volume, which characterize polycythemia vera, are uncommon in thrombocythemia.

## Treatment

Definitive therapy of primary thrombocythemia is directed at lowering the platelet count. <sup>32</sup>P-phosphate has been used successfully for this purpose for many years.<sup>559</sup> In appropriate doses, this isotope reduces the platelet count to desired levels with minimal side effects. Alkylating agents, such as melphalan<sup>513</sup> and busulfan,<sup>553</sup> and antimitotic drugs have also been widely used.<sup>560,574,575</sup> These agents may be administered on an intermittent schedule, use of the drug being discontinued when the platelet count falls to an approximately normal value and reinstituted when it rises again. A maintenance schedule may be more effective.<sup>513,542</sup> Because of the relatively long time required for the therapeutic effects of either <sup>32</sup>P or alkylating agents (two to six weeks), thrombocytapheresis has been recommended when an immediate reduction in the platelet count is required, eg, in serious hemorrhage or prior to a surgical operation.<sup>573</sup>

Anticoagulants such as heparin and coumarins (Chapter 39) have been widely used for the prevention and treatment of thromboembolic complications in thrombocythemia. Experience with these agents has been encouraging in some cases, disappointing in others.<sup>574</sup> When marked thrombocytosis is present, unusually high doses of heparin may be required.<sup>524,574</sup> Preliminary results with aspirin and other inhibitors of platelet function have been encouraging,<sup>578</sup> but these agents have not been thoroughly evaluated.

Splenectomy usually is contraindicated in thrombocythemia. In many subjects, uncontrollable rises in the platelet count and fatal complications have followed this procedure.<sup>513,514</sup> In certain instances, splenectomy



appeared to have "unmasked" previously latent thrombocythemia.<sup>513</sup>

## Reactive Thrombocytosis

Reactive thrombocytosis usually is moderate in degree, asymptomatic, short-lived, and responds to treatment of the underlying disorder (Table 34-8). Reactive thrombocytosis with the platelet counts above  $1,000 \times 10^9/l$  may be found in an occasional patient in whom two or more factors leading to thrombocytosis are concomitantly present, eg, inflammatory disease and iron deficiency.<sup>535</sup> In the vast majority of patients with reactive thrombocytosis, treatment directed at lowering the platelet count is not indicated.

### Post-splenectomy

Thrombocytosis is a predictable finding following splenectomy, but, contrary to the common impression, it does not necessarily develop immediately after this operation. A rising platelet count is first noted two to ten days after splenectomy, and usually reaches a peak one to three weeks later. Platelet counts in excess of  $1,000 \times 10^9/l$  are not uncommon.<sup>579</sup> The magnitude of post-splenectomy thrombocytosis is thus much greater than that which could result merely from the removal of the physiologic splenic platelet pool (page 385)<sup>331</sup>; a poorly understood increase in platelet production presumably is responsible. The observation that platelet levels of 5-hydroxytryptamine are subnormal in patients with reactive thrombocytosis following splenectomy remains unexplained.<sup>550,562</sup> Other evidence of platelet dysfunction has not been documented in such cases. The platelet count usually returns to normal levels in time, but several weeks or months may be required. Thromboembolic complications or hemorrhagic phenomena are rare in hematologically normal patients,<sup>531,548,549</sup> and there is little evidence to favor routine anticoagulation following splenectomy.<sup>579</sup>

When splenectomy is performed for the treatment of anemia, postoperative thrombocytosis often is marked, but the platelet count

usually falls to approximately normal levels if the anemia is completely corrected by the operation.<sup>549</sup> However, thrombocytosis often persists if anemia is not relieved.<sup>549</sup> In such patients, serious hemorrhage, thromboembolic manifestations, or both sometimes develop following splenectomy.<sup>526,548</sup> Treatment of the anemia by other measures may result in a reduction in the platelet count; if these measures are ineffective, and if the platelet count remains above  $1,000 \times 10^9/l$  and clinical manifestations result, therapy directed at lowering the platelet count, as described above, has been recommended.<sup>512</sup>

### Accelerated Hematopoiesis

Accelerated thrombopoiesis commonly is associated with accelerated erythropoiesis. Thus, increased platelet numbers are present in patients with various hemolytic anemias,<sup>556</sup> following acute blood loss,<sup>534,551,558</sup> and in some patients with secondary polycythemia associated with renal tumors<sup>518</sup> or congenital heart disease. The persistence of post-splenectomy thrombocytosis in anemic patients has been discussed above. Despite the evidence that "thrombopoietin" and erythropoietin are different substances<sup>554</sup>, these findings suggest that there is some interrelationship between erythropoietic and thrombopoietic control mechanisms<sup>549</sup> (page 386).

Thrombocytosis may be the result of "overshoot" or "rebound" phenomena<sup>569</sup> in several situations. Thus, increased platelet numbers are common findings in patients who have recovered from marrow suppression or thrombocytopenia of various types,<sup>568</sup> eg, suppression or thrombocytopenia due to alcohol,<sup>512</sup> pernicious anemia,<sup>568</sup> viral infections. Thrombocytopenia, followed by thrombocytosis when the drugs have been withheld, is a predictable effect of certain folic acid antagonists<sup>569,582</sup> and other antimitotic agents. Vincristine may specifically stimulate platelet production.<sup>573,576,577</sup> In tidal platelet dysgenesis (page 1098), thrombocytosis alternates with thrombocytopenia in a predictable cycle.

## Miscellaneous

Moderate thrombocytosis that persists for one to two weeks is commonly present one to two days after major *trauma* or *surgical operations*.<sup>519,527,572</sup> Thrombocytosis is common in pregnancy and the puerperium; this is discussed on page 388. In hemophilia, thrombocytosis may follow acute hemorrhage, but a moderate elevation of the platelet count frequently persists in the absence of bleeding.<sup>515</sup> *Iron-deficiency anemia* may sometimes be associated with thrombocytopenia (page 1097), but slight thrombocytosis is a more common finding.<sup>581</sup> Platelet counts are usually in the range of  $500 \times 10^9/l$ ; levels above  $1,000 \times 10^9/l$  have been documented<sup>528</sup> but these are rare. The contributory importance of acute bleeding in such patients remains controversial.<sup>533,558</sup> The platelets return to normal levels following specific therapy with iron.<sup>541</sup>

The association of thrombocytosis with various *neoplasms* has been recognized for many years. The magnitude of thrombocytosis appears to be unrelated to the presence or absence of metastases.<sup>545</sup> The highest platelet counts have been found in association with carcinomas of the breast and lung, and with Hodgkin's disease.<sup>558</sup> Thrombosis is a common complication in patients with cancer, but the etiologic significance of the thrombocytosis is uncertain because of the frequent coexistence of the "hypercoagulable" state (page 1237) and, in some patients chronic intravascular coagulation. In some patients with cancer or Hodgkin's disease, unexplained thrombocytosis may be an important clue to the presence of an occult neoplasm.<sup>530,531</sup>

Thrombocytosis is commonly associated with various acute and chronic *inflammatory* and *infectious processes* (Table 34-1). Experimental inflammation produces a similar thrombocytosis in animals,<sup>326,567</sup> but the mechanism by which this occurs remains obscure. Moderate thrombocytosis also is common in cirrhosis of the liver.<sup>558</sup> In various inflammatory disorders of the bowel<sup>564</sup> and in rheumatoid arthritis, thrombocytosis is a

frequent finding; it may provide a sensitive indicator of improvement or relapse in the basic disease.<sup>512</sup>

In many other chronic disorders, reactive thrombocytosis is a less consistent finding (Table 34-7), eg, various chronic renal diseases,<sup>558</sup> glycogen storage disease.<sup>510</sup>

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## Qualitative Disorders of Platelet Function

- Hereditary Disorders of Platelet Function
  - Thrombasthenia
  - Deficient Release Reaction
  - "Thrombopathy" (Deficient PF-3 Activity)
- Hereditary Platelet Dysfunction with Thrombocytopenia
- Miscellaneous Hereditary Forms
- Acquired Disorders of Platelet Function
  - Drug-Induced Platelet Dysfunction
  - Uremia
  - Platelet Dysfunction in Disorders of the Hematopoietic System
  - Effects of Fibrinogen Degradation Products on Platelet Function
  - Miscellaneous

DISORDERS of platelet function are difficult to classify because of the rarity of many forms, the numerous incompletely studied cases, and the considerable nosologic confusion which still surrounds this field. In the classification presented in Table 35-1, hereditary disorders are divided into three groups; namely, those in which platelet dysfunction is the sole abnormality (primary or "pure" forms); those in which mild to moderate thrombocytopenia is associated with abnormalities of platelet function; and a miscellaneous group, in which qualitative platelet dysfunction is but one facet of heritable disorders involving diverse organ systems. Platelet dysfunction also may complicate a wide variety of acquired disorders, but is seldom the sole abnormality. The discussion to follow will emphasize disorders in which abnormal platelet function appears to

be a major cause of bleeding. Information concerning disordered platelet function has accumulated at an unprecedented rate in the past decade and has been comprehensively reviewed.<sup>50,58,70,93,97,98,108</sup>

### Hereditary Disorders of Platelet Function

#### Thrombasthenia

It is now generally agreed that the term "thrombasthenia" (Glanzmann's thrombasthenia or disease, Glanzmann-Naegeli's disease, diacyclothrombopathia<sup>127</sup>) should be restricted to cases characterized by deficient ADP-induced platelet aggregation and deficient clot retraction.<sup>58,62</sup> Only when such diagnostic criteria are employed does this disorder emerge as a clearcut entity.

#### Pathophysiology

In thrombasthenia, the adhesion of platelets to collagen and the release reaction are normal, but the subsequent aggregation phase is lacking. Thrombasthenic platelets are refractory to the aggregating effects of ADP of either exogenous or endogenous origin (Fig. 35-1). This abnormality leads to a hemostatically inadequate platelet thrombus which presumably is the central feature in the pathogenesis of bleeding. Deficient platelet aggregation also may result in deficient plate-

**Table 35-1. Classification of the Qualitative Disorders of Platelet Function**

<b>I HEREDITARY</b>	
1	<i>Primary forms</i>
A	Thrombasthenia
B	Deficient release reaction
C	Thrombopathy* (deficient PF-3 activity)
2	<i>Varieties with thrombocytopenia</i>
A	The Bernard-Soulier syndrome
B	Thrombopathic thrombocytopenia
C	The Wiskott Aldrich syndrome
3	<i>Miscellaneous</i>
A	Hereditary afibrinogenemia
B	Heritable disorders of connective tissue
C	Other (mucopolysaccharidoses albinism glycogen storage disease)
<b>II ACQUIRED</b>	
1	<i>Drugs</i>
A	<i>Anti inflammatory agents</i> (aspirin phenyl butazone sulfinpyrazone indomethacin)
B	<i>Anidepressants</i> (chlorpromazine promethazine reserpine imipramine emytiriphen and congeners)
C	<i>Adrenergic blocking agents</i> (phenolamine dihydroergotamine)
D	<i>Miscellaneous</i> (ethanol, clofibrate dipyrindimol, nialamide diphenhydramine dextran and similar polymers papaverine carbencillin)
2	<i>Uremia</i>
3	<i>Disorders involving the hematopoietic system</i>
A	Paraproteinemias (macroglobulinemias multiple myeloma others)
B	Hemorrhagic thrombocythemia myelofibrosis polycythemia vera
C	Miscellaneous (acute and chronic leukemias ITP others)
4	<i>Miscellaneous</i>
A	Disorders associated with circulating fibrinogen degradation products (disseminated intravascular coagulation fibrinogenolysis liver disease)
B	Scurvy

have been found in many other cases. In vitro,  $Mg^{++}$  partially corrects the deficient clot retraction in some cases, but this cation is present in normal amounts in the plasma and platelets of patients with thrombasthenia.<sup>16,20</sup>

More recently, a deficiency of glutathione peroxidase was demonstrated in the platelets of three patients with thrombasthenia.<sup>63</sup> This abnormality was associated with elevated levels of reduced glutathione in two of the three patients. Deficiency of platelet glutathione reductase has been reported in an unrelated kindred with this disorder.<sup>107</sup> These observations suggest that the ability of thrombasthenic platelets to withstand "oxidative" stress is deficient. The relationship between these metabolic abnormalities and the pathophysiology of thrombasthenia has not as yet been clarified.

There is good evidence for a general defect in the platelet membrane in this disorder.<sup>18</sup> Thus, washed thrombasthenic platelets are unable to adsorb various cationic proteins, including factor XIIa, IgG and IgM immunoglobulins, and fibrinogen.<sup>3</sup> Subnormal levels of both membrane-adsorbed fibrinogen and that which is intrinsic to the platelet organelles have been demonstrated in several cases.<sup>33,109,110,164</sup> This protein appears to be an important cofactor for ADP-induced platelet aggregation (page 391), and may be involved in platelet adhesion to fibrin, a phenomenon essential for clot retraction (page 398). However, normal amounts of platelet fibrinogen have been documented in some patients with severe aggregation abnormalities,<sup>22,29</sup> and the failure of affected platelets to adhere to fibrin<sup>20,95</sup> has not been consistently observed.<sup>18</sup> Thrombasthenic platelets produce subnormal activation of factor XIII<sup>30,164</sup> and possibly plasminogen as well.<sup>18,158</sup> Deficiencies of an unidentified membrane antigen have been demonstrated in two patients with thrombasthenia.<sup>110</sup> These observations provide additional evidence that the membrane of thrombasthenic platelets is abnormal, but electron microscopy has revealed only variable and probably nonspecific ultrastructural abnormalities.<sup>18,21,23</sup>

let factor 3 (PF-3) availability and impaired orientation of platelets with respect to fibrin strands. The latter abnormality may underlie deficient clot retraction.<sup>62</sup>

Numerous studies of platelet enzymes and biochemical pathways that provide energy for platelet functions have revealed no consistent abnormalities.<sup>18,22,172</sup> Deficiencies of platelet glyceraldehyde phosphate dehydrogenase and pyruvate kinase were demonstrated in one study,<sup>52</sup> but normal levels of these enzymes

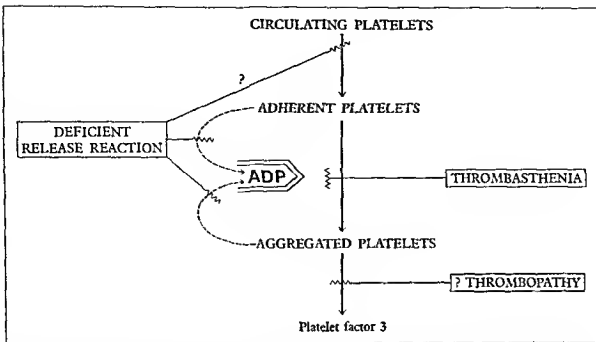


Fig 35-1. The pathophysiology of common disorders of platelet function. The sites of impairment of the processes of platelet adhesion and aggregation (solid arrows) and the release reaction (dashed arrows) in the various disorders (blocks) are indicated.

Although the platelet content of thrombosthenin is approximately normal as judged by qualitative techniques,<sup>62, 110, 172</sup> a deficiency of membrane-associated thrombosthenin (thrombosthenin S) has been demonstrated in the platelets of two patients with thrombasthenia by means of an immunohistochemical antibody staining method.<sup>11</sup> Other data suggest the presence of a qualitative abnormality of thrombosthenin.<sup>18a, 25</sup> In view of the hypothesized importance of this protein in both platelet aggregation and clot retraction (page 398), these observations, if confirmed, may provide a unifying concept of the pathophysiology of this disorder. However, it is probable that all cases presently included in this category are not the same,<sup>22</sup> and that a single molecular defect does not underlie all of the abnormalities which have been described.

### Clinical Features

More than 100 cases of thrombasthenia have now been reported. The disorder is inherited as an autosomal recessive trait and consanguinity is commonly present in

affected kindreds.<sup>118</sup> The disorder is clinically manifested by bleeding of the purpuric type. Common manifestations include epistaxis, menorrhagia, and gingival bleeding. Generalized ecchymoses may be striking.<sup>58</sup> In contrast to most other disorders of platelet function, spontaneous bleeding may be disabling, and post-traumatic and postoperative hemorrhage may be serious.

Heterozygotes are asymptomatic and laboratory studies usually show no abnormality, although deficient clot retraction as an isolated abnormality may be noted in some of these individuals.

### Laboratory Findings

The laboratory features of this disorder are summarized in Table 35-2. A prolonged bleeding time, deficient clot retraction, and the lack of ADP-induced platelet aggregation are characteristic and constant findings. Platelet aggregation by collagen, epinephrine, and other agents that act by releasing platelet-contained ADP is likewise deficient. The platelets are present in normal numbers

Table 35-2. Laboratory Findings in Disorders of Platelet Function

Test	Thrombasthenia	Deficient Release Reaction		Bernard-Soulier Syndrome
		Storage Pool Disease	Abnormal Release Mechanism	
Platelet count	U normal	U normal	U normal	Mild to moderate thrombocytopenia
Platelet morphology	Normal <sup>a</sup>	Normal microcytic <sup>c</sup> in some cases	—	Characteristic giant platelets
Bleeding time	M prolonged	V abnormality	U prolonged	U prolonged
Clot retraction	Deficient	Normal	Normal	Normal
Prothrombin consumption <sup>a</sup>	V abnormality	V abnormality	U abnormal	Abnormal <sup>g</sup>
Specific tests of PF-3 activity <sup>a</sup>	Abnormal <sup>†</sup>	V abnormality <sup>ab</sup>	V abnormality	V abnormality
Platelet retention in glass bead columns (glass "adhesion")	Reduced <sup>†</sup>	Reduced <sup>†</sup>	Reduced	—
Platelet aggregation by 5 $\mu$ M ADP	Deficient	Normal	Normal	Normal <sup>a</sup>
Platelet aggregation by "threshold" concentrations of ADP (0.2–1.5 $\mu$ M)	Deficient	V deficiency with subsequent disaggregation	V deficiency with subsequent disaggregation	Normal <sup>a</sup>
Platelet aggregation by dilute collagen suspensions and 5 $\mu$ M epinephrine	Deficient	Deficient <sup>†</sup>	Deficient	Normal <sup>a</sup>
Platelet aggregation by bovine fibrinogen (1.5 mg/ml)	V abnormality	Normal	Normal	Deficient
Storage nucleotide pool	Normal	Diminished	Normal	Normal <sup>h</sup>
Ancillary laboratory features	<sup>a</sup> Platelets appear discrete and rounded in stained smears <sup>†</sup> Not corrected by ADP Serum defects in TGT Platelet fibrinogen commonly decreased	<sup>c</sup> Corrected by ADP <sup>†</sup> Second wave of epinephrine-induced aggregation absent or markedly reduced Platelet dense bodies reduced in number	In vitro effects of aspirin are additive	Initial shape change lacking <sup>g</sup> One-stage techniques <sup>a</sup> Aggregation may be abnormally rapid <sup>h</sup> Corrected for increased platelet volume Ristocetin-induced platelet aggregation deficient

\*Results vary depending on exact technique employed  
Key: U = usually, M = markedly, V = variable



and are morphologically normal when viewed by light microscopy. In stained blood smears, however, the platelets remain discrete, round, and "isolated" as a result of deficient adhesion and aggregation,<sup>18,62</sup> which may explain reports of morphologic abnormalities. Clot retraction usually is absent, but may be detectable in an occasional subject.<sup>18</sup> Abnormalities in prothrombin consumption are variable, but the results of more sensitive tests of PF-3 activity usually are abnormal.<sup>158</sup> Correction is not obtained by the addition of exogenous ADP. Contact activation is subnormal in serum prepared from the blood of thrombasthenic patients,<sup>158</sup> and as a consequence the serum functions abnormally in the thromboplastin generation test (TGT). This has led to an erroneous diagnosis of factor IX deficiency in several cases. The results of other tests of coagulation are normal. Thromboelastographic tracings resemble those obtained in severe thrombocytopenia.<sup>18</sup>

### Treatment

There is no specific treatment. Corticosteroids and other nonspecific measures have been tried without effect. The transfusion of fresh blood or platelet concentrates has been beneficial in some patients<sup>171</sup> and disappointing in others.<sup>62</sup>

### Deficient Release Reaction

This disorder is the result of the inability of affected platelets to undergo a normal release reaction when physiologically stimulated. It is also known as "storage pool disease,"<sup>162</sup> the *Portsmouth syndrome*,<sup>114</sup> *primary platelet dysfunction*,<sup>181</sup> and "thrombopathia," the last in somewhat confusing contradistinction to "thrombopathy" or "thrombocytopenia"—disorders to be discussed below—in which deficient PF-3 activity is assumed to be the major abnormality. Similar but not identical defects in the release reaction apparently underlie platelet dysfunction which complicates a wide variety of acquired diseases, and that which is produced by numerous commonly used drugs.

### Pathophysiology

In this disorder, the platelets aggregate normally in response to exogenous ADP, but fail to release normal amounts of endogenous ADP (Fig. 35-1). As a consequence, the initial steps in platelet aggregation are not "re-enforced" or "amplified" by the release reaction and the resulting secondary wave of aggregation. The latter process apparently is essential for the rapid formation of a stable platelet plug (page 394).

In most cases, the basic defect appears to be a deficiency of storage nucleotides in affected platelets. Thus, Holmsen and Weiss<sup>72,73</sup> demonstrated an abnormally high specific activity of ATP and ADP in affected platelets isotopically labeled by incubation with <sup>14</sup>C-adenine. The conversion of labeled ATP into inosine monophosphate (IMP) and hypoxanthine proceeded normally. Similar abnormalities can be produced in normal platelets if their storage nucleotides are depleted by treatment with collagen. These data have been confirmed by others,<sup>59</sup> and suggest that in this disorder, which has been termed "storage pool disease," deficient ADP release is due to deficiency of available storage ADP and not to abnormalities in the pathways that supply energy to the release mechanism.<sup>73,163</sup> In other cases,<sup>73,162,165</sup> the storage nucleotide pool was normal, suggesting that the abnormalities in the release reaction were due to a different defect, which resembles that produced by aspirin. It is probable that several different biochemical abnormalities underlie hereditary deficiency of the release reaction.<sup>72,116</sup>

Deficiency of ADP release may be quantitated,<sup>63,160</sup> and in vitro gives rise to deficient platelet aggregation by agents that act by promoting the release of platelet ADP, eg, collagen, epinephrine, thrombin. Although deficient ADP release appears to be central to the pathogenesis of the bleeding, the uptake, storage, and release of substances other than ADP which are normally contained within the platelet also are subnormal, eg, serotonin, epinephrine, PF-4.<sup>57,73</sup>

Electron microscopy has revealed sub-

normal numbers of platelet "dense bodies" (Fig. 9-1, page 374) in the platelets of several patients with this disorder,<sup>59,162</sup> an observation which is consistent with the fact that this organelle is the major storage site for both ADP and serotonin. An identical abnormality is also present in albinism,<sup>162,169</sup> and may represent a truly specific ultrastructural manifestation of "storage pool disease."<sup>169</sup>

### Incidence and Clinical Features

The incidence of hereditary deficiency of the release reaction is unknown. If cases previously classified as "thrombopathy" are found to represent defects of the release reaction, as discussed below, the disorder would be relatively common.<sup>50</sup> The disorder appeared to be inherited as an autosomal dominant trait in several kindreds.<sup>22,23,100,168</sup> In others, a lifelong hemorrhagic diathesis was present but family members were unaffected.<sup>61,121</sup> Hereditary platelet dysfunction presumably due to deficiency of storage ADP also has been described in rats.<sup>154</sup>

The clinical picture is usually one of mucocutaneous bleeding of mild to moderate severity. Hematuria and epistaxis are common, but post-traumatic bleeding is seldom severe. Petechiae are relatively uncommon, and in many patients the only complaint was easy bruising.

### Laboratory Findings

The usual laboratory findings are summarized in Table 35-2. The bleeding time usually is moderately prolonged, but varies from normal<sup>100</sup> to markedly prolonged,<sup>22</sup> and fluctuates from time to time in the same patient.<sup>131</sup> The platelet count and results of coagulation studies are normal, clot retraction is normal. In several affected families the platelets were significantly smaller than normal.<sup>100,168</sup> The megakaryocytes are present in normal numbers and their morphologic appearance also is normal. Abnormalities of PF-3 activity have been demonstrated in most subjects, but are not invariably present.<sup>116,131</sup>

Platelet aggregation by high concentrations of exogenous ADP (5  $\mu$ M or higher) is normal. However, the amount of ADP required to produce the release of platelet-contained ADP and a "secondary" wave of aggregation ("threshold" concentration) has been found in this disorder to be two to three times higher than normal.<sup>63</sup> Consequently, concentrations of ADP that normally would suffice to induce optimal and irreversible aggregation (0.2 to 1.5  $\mu$ M) may produce slightly subnormal aggregation<sup>58</sup> that is rapidly followed by spontaneous deaggregation (Fig. 35-2).<sup>58,61,63,131</sup> Aggregation by collagen and epinephrine is deficient, and with the latter agent the second part of the normally biphasic aggregation curve is lacking (Fig. 35-2). Abnormalities in collagen-induced aggregation have been documented in apparently normal persons,<sup>150</sup> and may be minimized or abolished if concentrated collagen suspensions are used.<sup>111,131</sup> Serious diagnostic confusion may result from ingestion of aspirin or related drugs, which produce similar laboratory abnormalities.

### Treatment

There is no specific treatment for this disorder. The efficacy of platelet transfusions has not been adequately studied.<sup>131</sup>

### "Thrombopathy"

The term "thrombopathy" is usually employed in the sense proposed by Braunsteiner and Pakesch<sup>17</sup> to refer to platelet dysfunction manifested by defective coagulant activity of the platelets, i.e., deficient PF-3 activity (Fig. 35-1). Bowie and Owen<sup>14</sup> have divided these disorders into "deficit" thrombopathies, disorders in which PF-3 content of platelets is subnormal, and "functional" thrombopathies wherein the "availability" or "release" of PF-3 is deficient, although the total content is normal. Hereditary and acquired forms have been described in both categories. Other variants,<sup>16</sup> and "compound" defects, representing combinations of thrombopathy,

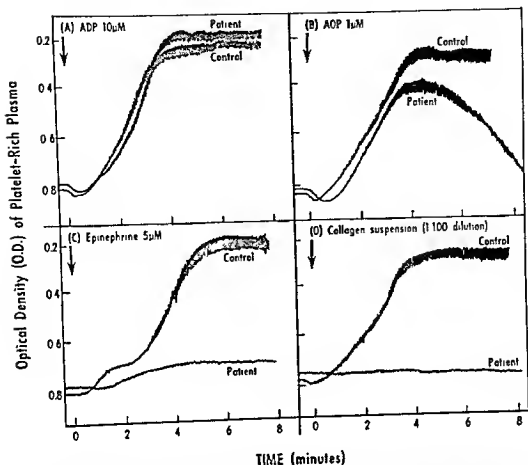


Fig. 35-2. Aggregometer tracings in a patient with deficient release reaction. Aggregating agents were added at zero time. Note the disaggregation observed with low ADP concentrations (B), the absence of the secondary wave of epinephrine-induced aggregation (C), and the absence of collagen-induced aggregation (D). The patient was a 31 year old man with partial albinism. Ancillary findings included characteristic pigmented reticulum cells in the bone marrow, and abnormally small platelets.

thrombasthenia, and deficiencies of factors VIII<sup>36</sup> and IX, also have been described.<sup>14</sup>

Bleeding manifestations are mild, and serious hemorrhage is rare. In addition to abnormal prothrombin consumption and deficient PF-3 activity of the platelets in various *in vitro* systems, the bleeding time may be prolonged but often is normal. Clot retraction is normal. There is no specific treatment of established value, although corticosteroids have produced improvement in some cases.

The status of "thrombopathy" as a specific form of platelet dysfunction may now be questioned.<sup>50,70,97</sup> The mechanism by which deficient PF-3 activity alone leads to a prolonged bleeding time and clinical bleeding remains unclear. *In vitro* studies by Hardisty

and Hutton<sup>60</sup> and others<sup>2,160</sup> have established that PF-3 activity normally is proportional to and dependent upon platelet aggregation in earlier steps. A large number of drugs that alter the platelet release reaction also produce abnormalities in PF-3 activity,<sup>108</sup> and studies of many of the acquired forms of platelet dysfunction previously termed "thrombopathy" have revealed clearcut abnormalities in platelet aggregation, eg, in uremia, myelofibrosis. "Thrombopathy" would thus appear to be closely related to if not identical with deficient release reaction.<sup>60,70,160</sup> The entire subject has been confused by the previously discussed inadequacies in terminology and in available methods for measurement of "platelet factor 3"<sup>97,98</sup> (page 397).

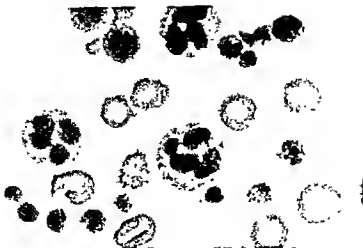


Fig. 35-3. Giant platelets in the Bernard-Soulier syndrome  $\times 1200$ , Wright's stain (From Bihelli et al.<sup>8</sup> courtesy of the authors and the Annals of the New York Academy of Sciences.)

### Hereditary Platelet Dysfunction with Thrombocytopenia

#### Bernard-Soulier Syndrome

The Bernard-Soulier syndrome is a relatively rare disorder that was first described in 1948 under the name "*dystrophie thrombocytaire hémorragique congénitale*."<sup>7</sup> It is characterized by giant platelets, mild thrombocytopenia, bleeding out of proportion to the reduction in platelet numbers, and variable abnormalities in PF-3 activity. The disorder is inherited as an autosomal recessive trait, and consanguinity is common in reported kindreds.<sup>7,93</sup> Heterozygotes are almost invariably asymptomatic, but, in some, mild deficiency of PF-3 activity and "giant" platelets are present. The clinical picture is one of moderate to severe bleeding manifestations of the purpuric type, including bruising, epistaxis, and menorrhagia. There is no known therapy, and splenectomy has been ineffective.

The morphologic abnormalities of the platelets are the most consistent and striking feature of the disorder (Fig. 35-3). The platelets range in size up to  $8\ \mu\text{m}$  in diameter,

have a relatively dense granulomere, and have been described as "lymphocytoid."<sup>93</sup> The megakaryocytes are normal or increased in number, but reveal no characteristic morphologic abnormalities.

The nature of platelet dysfunction in this disorder has not been well characterized. The bleeding time is prolonged, but clot retraction, and platelet aggregation by ADP and collagen are normal.<sup>8,53,93</sup> Platelet aggregation by bovine fibrinogen<sup>8,46a</sup> and by Ristocetin<sup>41,96</sup> is deficient.<sup>8</sup> In two cases, the initial shape change normally produced by ADP was not evident.<sup>8</sup>

Grøttum and Solum<sup>53</sup> demonstrated reduced electrophoretic mobility of affected platelets, which they attributed to deficiency of sialic acid in the membrane. These observations may explain the "hyperaggregability" of giant platelets which has been frequently observed.<sup>8,93</sup> No other abnormalities in platelet biochemistry or characteristic ultrastructural features have been described.<sup>112</sup> In two reports,<sup>37,53</sup> biphasic platelet survival curves suggested the presence of two populations of platelets, i.e., the "giant" forms which survived only 0.5 to 0.6 day, and smaller platelets which had a normal survival time.

## "Thrombopathic" Thrombocytopenia

"Thrombopathic" thrombocytopenia is a disorder that resembles the Bernard-Soulier syndrome in most respects, but is inherited as an autosomal dominant trait<sup>69,89,92,123</sup> Platelet counts of 20,000 to 80,000/ $\mu$ l with numerous "giant" forms have been reported, in spite of normal numbers and normal morphologic appearance of the megakaryocytes.<sup>89</sup> In one kindred<sup>92</sup> the morphologic abnormalities of the platelets antedated the thrombocytopenia, and bleeding ameliorated at the time of adolescence. Subtle variations in the appearance of the abnormal platelets have been noted,<sup>92</sup> and ultrastructural studies have revealed a reduced number of alpha granules.<sup>89,157</sup> In one kindred,<sup>3</sup> the total nucleotide content of the "giant" platelets was normal when results were corrected for platelet volume, but other studies demonstrating deficient collagen-induced aggregation and PF-3 activity suggested an abnormality of the release reaction.

Giant platelets and moderate thrombocytopenia apparently are common in certain populations of Mediterranean extraction.<sup>91</sup> They may be associated with other hereditary or congenital syndromes, eg, monoclonal gammopathy,<sup>183</sup> autosomal dominant nephritis and deafness.<sup>43</sup>

## Wiskott-Aldrich Syndrome

In addition to thrombocytopenia and specific abnormalities of immunologic defense, the Wiskott-Aldrich syndrome (Chapter 44) is characterized by qualitative abnormalities of platelet function. Platelets from affected persons are smaller than normal and, under the electron microscope, reveal deficiencies in the number of alpha granules and other ultrastructural abnormalities.<sup>54</sup> Platelet aggregation induced by epinephrine, ADP, and collagen is deficient, and the platelet storage pool of adenine nucleotides is subnormal. Studies by Kuramoto et al<sup>88</sup> demonstrated an interesting metabolic defect in the platelets in persons with this disorder. The abnormality

was manifested by deficient CO<sub>2</sub> production in the citric acid cycle following exposure of platelets to latex particles and aggregating agents. Kuramoto and associates suggested that a similar defect in the metabolic response of macrophages could underlie abnormalities in antigen processing. The major discussion of this syndrome will be found on page 1387.

Minor abnormalities in PF-3 activity have been reported in some cases of the *May-Hegglin anomaly*<sup>86</sup> (page 1322).

## Miscellaneous Hereditary Forms

### Hereditary Afibrinogenemia

In contrast to most coagulation disorders, hereditary afibrinogenemia (Chapter 37) is usually associated with a prolonged bleeding time. As discussed elsewhere (page 392), fibrinogen apparently is required for ADP-induced platelet aggregation, and severe deficiencies of this factor produce a secondary abnormality in platelet function manifested *in vitro* by deficient platelet aggregation with low concentrations of ADP,<sup>140</sup> markedly defective adhesiveness of platelets to glass,<sup>166</sup> and variable abnormalities in PF-3 activity.<sup>55,78</sup> All of these abnormalities are corrected by fibrinogen both *in vivo* and *in vitro*. Defective hemostasis in this disorder may also result from a lack of the stabilizing effect of fibrin on the platelet thrombus.<sup>128</sup>

### Heritable Disorders of Connective Tissue and Mucopolysaccharidoses

Abnormally large platelets and laboratory data suggesting abnormalities in ADP release have been described in patients with various heritable disorders of connective tissue, including the Marfan syndrome, osteogenesis imperfecta,<sup>67</sup> and the Ehlers-Danlos syndrome<sup>44,45,84</sup> and in those with mucopolysaccharidoses. Many of these patients had no history of abnormal bleeding, and in others the results of platelet function studies were normal despite the presence of large platelets.

Various coagulation abnormalities may be associated with these disorders,<sup>45</sup> and bleeding may also result from defective vascular support (Chapter 38). Platelets do not adhere normally to collagen obtained from patients with the Ehlers-Danlos syndrome.<sup>62a</sup>

### Albinism

A mild to moderate hemorrhagic disorder associated with prolongation of the bleeding time has been reported in several patients with albinism. In some, abnormal ceroid-like pigmentation of the leukocytes<sup>169a</sup> and reticulum cells of the bone marrow was observed (Hermansky-Pudlak syndrome).<sup>68,154</sup> In adequately studied cases, deficient storage of ADP, reduced numbers of platelet "dense bodies,"<sup>102,160</sup> and platelet function abnormalities characteristic of deficient ADP release were found<sup>58,59,93</sup> (Fig. 35-2).

### Glycogen Storage Disease

A mild bleeding diathesis with prolonged bleeding time, deficient PF-3 activity, and abnormalities of platelet aggregation compatible with defective ADP release has been reported in patients with glycogen storage disease.<sup>37a,49,66,113a</sup> Platelet dysfunction was thought to be due to interaction of the platelets with factors in the plasma, possibly lipids.

### Unclassified Varieties

As is the case with the hereditary coagulation disorders, a large literature has accumulated concerning isolated kindreds or single case reports that appear to represent unique disorders of platelet function. Under the name "gray platelet syndrome", Raccuglia<sup>126</sup> described a disorder manifested by lifelong purpura. The patient initially presented with moderate thrombocytopenia, which was successfully treated by splenectomy. Prolongation of the bleeding time and poor clot retraction persisted despite normal platelet numbers. The platelets revealed an almost total lack of granules, and a peculiar gray

color in Wright-stained blood smears. Biochemical studies of affected platelets demonstrated moderate reduction in platelet ATP and in extractable phosphatides, but aggregation with ADP, epinephrine and collagen, and PF-3 activity as measured in the TGT were normal. Family studies failed to provide evidence for a hereditary origin.

Many other reports appear to represent minor variants of the more common forms already discussed. Thus, "essential athrombia"<sup>77,142</sup> refers to a disorder that resembles thrombasthenia in all respects, except for the presence of detectable clot retraction. A structural defect in collagen obtained from one patient appeared to underlie deficient platelet adhesion to collagen fibers.<sup>19</sup> Adhesion to collagen was deficient in several other patients affected with a disorder which resembled deficient release reaction in most other respects.<sup>22,71,116,131</sup> Quite similar cases have also been reported as "mild" thrombasthenia.<sup>34</sup> Whether or not these cases represent unique variants is unclear. Numerous reports of "hybrid" forms of platelet dysfunction and of genetic variants of well-recognized forms are not entirely convincing.<sup>120,121,122,130,134,137,145</sup>

## Acquired Disorders of Platelet Function

### Drug-Induced Platelet Dysfunction

A great many chemically and biologically active substances can be shown to inhibit platelet function, and much basic information has been obtained from the study of the *in vitro* effects of agents such as structural analogs of ADP and supraphysiologic concentrations of various drugs.<sup>108</sup> These data are briefly discussed in Chapter 9. The present discussion will be limited to drugs in common use which impair platelet function in therapeutic concentrations. Such drugs are listed in Table 35-1. The rapidly expanding body of knowledge concerning the influence of drugs on platelet function has been reviewed.<sup>70,97,104,108</sup>

## Mechanism of Action

In ordinary therapeutic doses aspirin will impair the platelet release reaction and secondary platelet aggregation for approximately one week.<sup>16,161,167,168</sup> This has been attributed to the irreversible acetylation of membrane proteins.<sup>1</sup> A similar mechanism cannot explain the action of the pyrazole compounds, since their effects are readily reversible and depend on continuously maintained blood levels.<sup>108</sup> Aspirin does not affect the storage nucleotide pool, but has been shown to impair glucose uptake, lactate production, and the transformation of ATP to IMP and hypoxanthine. These findings indicate an impairment of the pathways involved in the production or transfer of energy required in the release reaction.<sup>4,40</sup> Aspirin<sup>111</sup> and indomethacin<sup>118</sup> inhibit the production of prostaglandin  $E_2$  by platelets, an effect which may underlie the inhibition of the release reaction produced by these anti-inflammatory agents.<sup>16,99</sup> The antidepressants and some antihistamines are thought to "stabilize" mitochondria and biologic membranes,<sup>108</sup> whereas alpha-adrenergic blocking agents prevent the facilitation of ADP-induced aggregation by various sympathomimetic amines<sup>108</sup> (page 1102). The methyl xanthines act by inhibiting platelet phosphodiesterase, which leads to an increase in platelet levels of cAMP (Fig. 9-8, page 395). Dextran and related polymers presumably act by coating the platelets and altering the surface change.<sup>159</sup> Glyceryl guaiacolate is a structural analog of adenosine and inhibits ADP-induced platelet aggregation<sup>139</sup>; many other drugs produce similar effects in supraphysiologic concentrations.<sup>104,108</sup> Ethanol inhibits both primary and secondary ADP-induced aggregation.<sup>67a</sup> Most of the drugs included in Table 35-1 produce vasodilatation,<sup>99</sup> thus suggesting that they act on the contractile apparatus of the platelet.<sup>97</sup>

## Significance of Drug-Induced Platelet Dysfunction

The abnormalities produced by the drugs listed in Table 35-1 are quite variable, but

resemble in most respects those associated with hereditary deficiency of the release reaction as described above (Table 35-2). The bleeding time may be slightly prolonged, but often is not above the limits of normal. The results of more sensitive tests such as collagen and epinephrine-induced platelet aggregation may be abnormal and thus may lead to serious diagnostic confusion.

In otherwise normal persons, the impairment of platelet function produced by drugs is usually of no clinical significance, although a mild purpuric disorder may be observed in association with chronic aspirin ingestion. To the contrary, in patients with hereditary coagulation disorders, in uremic patients, and in patients receiving heparin or coumarin anticoagulants, impairment of platelet function by drugs may remove one of the remaining hemostatic defenses and result in serious bleeding.<sup>81</sup> In pregnant women, aspirin may cross the placenta and produce significant impairment of platelet function in the fetus.<sup>9</sup> Analgesics which are presumably safe in these patients include acetaminophen and propoxyphene.<sup>50</sup> Of perhaps greater long-term significance is the possible usefulness of drugs that impair platelet functions in the treatment and prevention of thrombosis (Chapter 39).<sup>97,108,161</sup>

## Uremia

Although the hemostatic defect in uremia often is complex and may include thrombocytopenia (page 1102) and minor coagulation abnormalities, it is probable that platelet dysfunction is the most consistent and clinically important feature.<sup>97</sup> Uremia was one of the first "acquired thrombopathies" to be described. The basic defect appears to be in the release reaction. Thus, deficient collagen<sup>125</sup> and epinephrine-induced aggregation,<sup>74</sup> impaired glass "adhesiveness,"<sup>42,132</sup> and a lack of the secondary wave of ADP-induced aggregation and subsequent disaggregation<sup>76</sup> have been demonstrated in several studies. The bleeding time is variably prolonged and prothrombin consumption and PF-3 activity usually are deficient.

There is ample evidence that these abnormalities are produced by a dialyzable factor in the plasma, but the nature of the responsible substance remains controversial.<sup>147,156</sup> In vitro, the effects of creatinine are variable and inconsistent, but urea,<sup>38a</sup> guanidosuccinic acid,<sup>74,75</sup> a metabolite of urea, and various phenols and phenolic acids<sup>124,125</sup> produce many of the characteristic laboratory abnormalities. Hypermagnesemia, which is present in many uremics, may be involved.<sup>38</sup> It is probable that numerous metabolites that accumulate in the plasma in uremia may separately or collectively affect platelet function.

Bleeding may be severe in the uremic patient. Widespread ecchymoses and intractable slow gastrointestinal bleeding are common, and large hemorrhages into serous cavities and into muscles may occur. Hemodialysis and peritoneal dialysis are of temporary therapeutic value. It should be pointed out that uremia is frequently a complicating factor in other complex hemorrhagic disorders, eg, multiple myeloma, severe liver disease, chronic forms of intravascular coagulation.

### Platelet Dysfunction in Disorders of the Hematopoietic System

#### The Paraproteinemias

The bleeding diathesis which may complicate the various paraproteinemias (Chapter 53) is exceedingly complicated. Factors that may play a role either alone or in combination include thrombocytopenia, fibrinolysis, various coagulation abnormalities, including those caused by specific and nonspecific inhibitors, vascular infiltration, and uremia.<sup>117</sup>

Platelet dysfunction can frequently be demonstrated, and is most common in macroglobulinemia.<sup>105,115</sup> Deficient PF-3 activity, reduced platelet adhesiveness,<sup>117</sup> and variable abnormalities of aggregation<sup>140</sup> have been documented and have been attributed to coating of the platelet membrane with abnormal protein.<sup>115</sup> In contrast to normal immunoglobulins, purified IgG fractions from patients with multiple myeloma, and IgM fractions from patients with macroglobulinemia, do not restore the aggregability

of washed normal platelets.<sup>5</sup> The displacement of normal proteins from the plasmatic atmosphere by aberrant proteins of various types may explain the aggregation abnormalities.

The presence of marked hyperglobulinemia may produce unique in vitro abnormalities. Thus, in one patient with multiple myeloma, deficient clot retraction not attributable to either thrombocytopenia or platelet dysfunction resulted from coating of fibrin strands by abnormal plasma proteins.<sup>90</sup> A similar interaction between abnormal proteins and collagen fibers was the apparent cause of abnormal platelet function in another patient.<sup>155</sup> The latter phenomenon may significantly impair hemostasis in vivo, since the bleeding time in these disorders often is normalized by plasmapheresis.<sup>51</sup>

#### Hemorrhagic Thrombocythemia, Myelofibrosis, and Polycythemia Vera

Bleeding has long been recognized as a complication of these disorders.<sup>56</sup> Common bleeding manifestations include ecchymoses, epistaxis, gastrointestinal bleeding, and a propensity to serious hemorrhage following minor trauma or surgical procedures. Petechiae are uncommon.

Hemorrhagic ("idiopathic") thrombocythemia (Chapter 34) is characterized by marked elevations of the platelet count, the magnitude of which appears to be correlated with the prolongation of the bleeding time and the severity of hemorrhage.<sup>103</sup> Large and morphologically abnormal platelets may be seen in stained smears, and deficiencies of PF-3 activity<sup>149,163</sup> and diminished platelet retention<sup>35</sup> have been reported. In contrast, none of these abnormalities was found in "reactive" or "secondary" thrombocytosis, conditions in which bleeding is uncommon.<sup>103</sup> However, platelet content and uptake of serotonin were reported to be subnormal both in patients with hemorrhagic thrombocythemia and in those with "secondary" thrombocytosis following splenectomy.<sup>103</sup>

In some cases,<sup>143</sup> a curious constellation of abnormalities was described which included normal PF-3 activity despite deficient



ADP-induced aggregation, and normal collagen-induced aggregation together with markedly diminished epinephrine-induced aggregation. In others, data consistent with a deficient release reaction have been obtained.<sup>148</sup> The inhibitory effects of high platelet concentrations in the TGT<sup>143</sup> and the inconstant and minor coagulation abnormalities that have been reported<sup>17,135</sup> are of doubtful pathophysiologic significance.

In myelofibrosis (Chapter 57), numerous reports of "thrombopathy"<sup>39,162</sup> have substantiated the presence of deficient PF-3 activity; an abnormality that may be present even in the absence of thrombocytosis.<sup>39</sup> Similar observations have been reported in polycythemia vera,<sup>79,82,163</sup> and preliminary evidence suggests that the release reaction is abnormal in this disorder.<sup>27</sup> The abnormalities in clot retraction, the phenomenon of "red cell fallout" and the "fragile" clots that have been described in polycythemia vera may be artifacts resulting from the markedly increased red cell mass.<sup>136</sup> In chronic myelocytic leukemia, an abnormal substance in the plasma, possibly an adenosine deaminase, produces abnormalities in platelet function.<sup>117a</sup>

The clinical importance of platelet dysfunction in hemorrhagic thrombocytopenia, myelofibrosis, polycythemia vera and chronic myelocytic leukemia remains unclear. Thromboembolic complications are common. The role of platelets and functional abnormalities thereof in the pathogenesis of thrombosis is currently under intensive study<sup>97,108,161</sup> (Chapter 39). Therapy directed at the underlying disorder may produce a parallel improvement in the bleeding and thromboembolic manifestations. Platelet transfusions have been helpful in some cases.<sup>27</sup> The treatment of thrombocytosis is discussed on page 1106.

#### Effects of Fibrinogen Degradation Products on Platelet Function

Fibrinogen degradation products (FDP) are protein fragments that result from the proteolytic cleavage of fibrin or fibrinogen by plasmin (Fig. 10-8, page 437). In vitro, FDP

in relatively high concentrations impair both ADP-induced aggregation and the release reaction.<sup>6,85</sup> In some studies, the large fragments X and Y ("early" FDP) appeared to be the more potent in this respect,<sup>80,85</sup> whereas in others much smaller dialyzable fragments were the more active.<sup>94,144,151</sup> It has been suggested that FDP are adsorbed to the platelet surface in competition with plasma fibrinogen, which is important as a cofactor of ADP.<sup>85</sup>

Impairment of platelet function has been correlated with in vivo levels of FDP in experimentally induced hyperfibrinolysis in dogs<sup>86</sup> and in man following therapeutic defibrination with Arvin.<sup>119</sup> The levels of FDP also parallel the severity of bleeding in many patients with cirrhosis of the liver.<sup>152</sup> In cirrhosis, inadequate hepatic clearance of plasminogen activators (page 1205) frequently results in persistent fibrinogenolysis and high levels of FDP. Impairment of platelet functions by FDP may thus be an important contributory cause of bleeding in fibrinogenolysis, disseminated intravascular coagulation, and decompensated liver disease (Chapter 38).

Fibrin monomers and complexes thereof<sup>108</sup> and polymerizing fibrin<sup>113</sup> also are often present in the plasma of cirrhotics, possibly as the result of low-grade intravascular coagulation.<sup>151</sup> In vitro, these derivatives accelerate ADP-induced platelet aggregation, and in vivo may lead to sequestration of platelets and thrombocytopenia (page 438). Thus, in decompensated liver disease, and possibly in intravascular coagulation as well, both the number and functional competence of the platelets may vary, depending on a continuously shifting balance between inhibitory and acceleratory effects of various fibrinogen derivatives.<sup>151</sup>

#### Miscellaneous

Platelet dysfunction has been reported in various other hematologic disorders<sup>26,98,129</sup> including idiopathic thrombocytopenic purpura,<sup>10,32</sup> acute and chronic leukemia,<sup>24,33a,48,163</sup> systemic lupus erythemato-

sus,<sup>163</sup> pernicious anemia,<sup>146</sup> and factor VIII deficiency.<sup>26,39</sup> In most cases, platelet dysfunction appeared to be a minor or inconsistent feature of the disorder.

Significant bleeding may occur in scurvy, and has traditionally been attributed to vascular abnormalities (Chapter 36). Significant platelet dysfunction has been reported in some cases of scurvy in man<sup>31,170</sup> and in scorbutic animals.<sup>12</sup> When performed, aggregation studies have suggested an abnormality in the release reaction.<sup>68</sup> Poorly defined abnormalities in platelet function also have been described in patients with congenital heart disease<sup>66,67,101</sup> and homocystinuria.<sup>173</sup>

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## *Bleeding Disorders Caused by Vascular Abnormalities*

Autoimmune Vascular Purpuras  
Allergic Purpura  
Drug-Induced Vascular Purpura  
Purpura Associated with Infections  
Structural Malformations of Vessels and  
Perivascular Tissues  
Hereditary Hemorrhagic Telangiectasia  
Hereditary Disorders of Connective Tissue  
Acquired Disorders of Connective Tissue  
Miscellaneous Vascular Purpuras  
Autoerythrocyte Sensitization and Related  
Disorders  
Purpura in Association with Paraproteine-  
mias  
Purpura Simplex and Related Disorders  
Purpura Associated with Skin Diseases  
and Other Conditions

knowledge of the basic pathophysiology remains fragmentary. Laboratory techniques for the study of vascular disorders are grossly inadequate, and the diagnosis usually is made from the appearance of the lesions and the clinical findings (Table 36-2), which fortunately often are characteristic. The results of laboratory tests of hemostasis and blood coagulation usually are within normal limits, and even painstaking pathologic study of biopsy material often reveals nonspecific findings. Although a wide variety of therapeutic agents has been tried, treatment remains purely empiric and usually is ineffective.

### **Autoimmune Vascular Purpuras**

#### **Allergic Purpura**

The term "allergic purpura" (anaphylactoid purpura, Henoch-Schönlein purpura) refers to a syndrome characterized by a relatively distinctive purpuric eruption in association with various constitutional and localized symptoms.<sup>1,28,41</sup> Such a broad clinical definition has proved useful, but encompasses a rather varied group of cases, and it is probable that the syndrome of allergic purpura represents one of the more benign clinical expressions of diffuse "autoimmune" vascular

**D**ISORDERS in which the major cause of bleeding is presumably an abnormality of the vessels or their supporting tissues are summarized in Table 36-1. Bleeding has often been attributed to such vascular disorders, primarily because no abnormalities of the platelets or of the coagulation mechanism could be demonstrated. A number of disorders previously consigned to this "diagnostic wastebasket" are now known to be associated with specific abnormalities of coagulation or platelet function, eg, uremia, hereditary deficiency of the release reaction, von Willebrand's disease. However, in the majority of the disorders to be discussed,

**Table 36-1. Purpuras Due Mainly to Vascular Abnormalities**

<b>I AUTOIMMUNE VASCULAR PURPURAS</b>	
1	The allergic purpuras
2	Drug induced vascular purpura (iodides, <sup>127</sup> belladonna, atropine quinine <sup>151</sup> procaine penicillin <sup>53</sup> phenacetin aspirin, <sup>122</sup> merbaphen, chloral hydrate and other sedatives, <sup>110</sup> various sulfonamides, coumarins others <sup>54</sup> )
3	Purpura fulminans (page 1219)
<b>II INFECTIONS</b>	
1	Bacterial (meningococcemia and septicemia due to other organisms <sup>144</sup> typhoid fever, scarlet fever <sup>23</sup> diphtheria tuberculosis <sup>20</sup> endocarditis <sup>145</sup> bacterial products <sup>125</sup> others)
2	Viral (smallpox influenza measles others)
3	Rickettsial (Rocky Mountain spotted fever typhus others)
4	Protozoal (malaria <sup>153</sup> )
<b>III STRUCTURAL MALFORMATIONS</b>	
1	Hereditary hemorrhagic telangiectasia
2	Hereditary disorders of connective tissue (Ehlers-Danlos syndrome osteogenesis imperfecta pseudoxanthoma elasticum)
3	Acquired disorders of connective tissues (scurvy corticosteroid purpura Cushing's disease, senile purpura, cachectic purpura)
<b>IV MISCELLANEOUS</b>	
1	Autoerythrocyte sensitization and related syndromes (DNA hypersensitivity cutaneous hyperactivity to hemoglobin psychogenic purpura, vicarious bleeding stigmata)
2	Paraproteinemias (page 1152) (hyperglobulinemic purpura cryoglobulinemic purpura Waldenström's macroglobulinemia, others)
3	Purpura simplex and related disorders (orthostatic and mechanical purpura, factitious purpura)
4	Purpura in association with certain skin diseases (annular telangiectatic purpura, angioma serpiginosum Schamberg's disease, pigmented purpuric fichenoid dermatitis)
5	Others (blood borne tumor emboli <sup>154</sup> snake venoms, <sup>132</sup> hemochromatosis <sup>155</sup> amyloidosis <sup>147</sup> other chronic diseases)

injury. A similar process presumably underlies a broad spectrum of poorly understood disorders including the inflammatory erythemas,<sup>142</sup> various collagen vascular disorders,<sup>42,142</sup> certain "immune complex" diseases,<sup>44</sup> and various forms of vasculitis<sup>42</sup> and allergic granulomatosis.<sup>142</sup>

## Etiology and Pathophysiology

The basic lesion is an inflammatory process, involving mainly the capillaries and arterioles, which results in perivascular infiltration and serosanguineous effusions into subcutaneous, submucous, and subserous tissues. Such effusions produce, in addition to purpura, the various localized and generalized manifestations. It is generally assumed that the disorder is the result of an autoimmune process, but the evidence for this is scanty and indirect. Similar lesions can be produced experimentally in rats by the sequential injection of agar and epinephrine,<sup>52</sup> and in dogs<sup>17</sup> and guinea pigs by administration of rabbit antisera against guinea pig vascular endothelium. Attempts to demonstrate a humoral antibody serologically have been unsuccessful, but in one patient in whom allergic purpura was associated with the administration of quinine, readministration of the offending drug produced transient thrombocytopenia, increased capillary fragility, and erythrophagocytosis.<sup>19</sup>

A few cases have been described in which hypersensitivity to certain foods seemed to be the cause of the disorder,<sup>4</sup> but in only a minority is the evidence convincing. Eggs, milk,<sup>35</sup> chocolate, wheat, and beans have been implicated most often.<sup>1,2,33</sup> Hypersensitivity to cold,<sup>46,49</sup> insect bites,<sup>14</sup> and rarely to drugs<sup>18,19,22</sup> also has been associated.

The frequency with which the onset of allergic purpura follows various infectious diseases has led to extensive study of the etiologic importance of infection. The streptococcus has been implicated most often, largely on the basis of epidemiologic studies suggesting that a common etiologic agent is involved in allergic purpura and in acute post-streptococcal glomerulonephritis.<sup>24</sup> However, antistreptolysin O (ASO) titers and attempts to isolate beta-hemolytic streptococci have failed to provide evidence as clear as there is in rheumatic fever and nephritis, that these organisms are causative factors.<sup>5,15,54,58</sup> The importance of hypersensitivity to bacterial products was suggested in one study, in which delayed skin hypersensitivity to vari-

**Table 36-2. The Appearance and Associated Clinical Features of Various Forms of Purpura**

<i>Disorder</i>	<i>Appearance*</i>	<i>Distribution</i>	<i>Associated Features</i>
Allergic purpura	Highly variable: small ecchymotic lesions on erythematous maculopapular base; urticarial lesions; bullae, ulcers in some cases	Symmetric, proximal: extremities, legs and buttocks	Lesions pruritic; ancillary joint and abdominal symptoms; generalized bleeding absent
Purpura fulminans	Large symmetrical spreading ecchymoses, circumscribed skin infarcts; petechiae uncommon	Often symmetric: distal extremities, genitalia	Symmetric gangrene of digits and distal extremities; fever, severe prostration; generalized bleeding and coagulation abnormalities common
Scurvy	Petechiae: often around hair follicles; ecchymoses: large subcutaneous hematomas	Often symmetric: "saddle" area of thighs and buttocks	Lassitude, pain in limbs; evidence of periosteal hemorrhage in children; generalized bleeding; other avitaminoses and positive reaction to tourniquet test common
Autoerythrocyta sensitization	Solitary: often large spreading purplish to reddish ecchymoses on an erythematous edematous base	Proximal extremities: antero-lateral thighs and legs; abdomen: rare on back	Prodromata: lesions often painful and tender; nausea, vomiting and other generalized symptoms; hysterical and neurotic symptoms; menorrhagia, hematuria, and epistaxis common; positive reaction to skin test
Thrombocytopenic purpura	Purple to black petechiae; superficial ecchymoses of varying size and shape	Anywhere: most common in dependent areas, sites of venous constriction and "pressure" points	Generalized bleeding from mucosal surfaces common

\*Purpuric lesions appear and regress in crops; are not elevated, do not blanch on pressure

ous bacterial vaccines, including those prepared from streptococci, was demonstrated in patients with allergic purpura but not in controls.<sup>40</sup> It also has been suggested that allergic purpura is a form of "immune complex" disease involving IgA antibody and an unidentified antigen,<sup>55</sup> but the evidence for this hypothesis is not altogether convincing.

The presence of fibrin within the glomerular deposits led to the suggestion that diffuse intravascular coagulation was involved in the pathogenesis of allergic purpura,<sup>56</sup> a hypothesis which is inconsistent with other data demonstrating normal fibrinogen turnover

rates and the absence of fibrinogen degradation products.<sup>55</sup>

### Incidence

The syndrome is seen most often in children, less commonly in adolescents and young adults, and rarely in older persons. The average age of onset in two series was 5.6 years<sup>37</sup> and 5.2 years,<sup>47</sup> respectively. While the condition is uncommon in children less than two years of age, in a series of 139 patients the age range was from 10 months to 12½ years.<sup>37</sup> Males predominate in a ratio



of 3:2.<sup>18,54</sup> In Great Britain, spring and autumn peaks of incidence have been observed.<sup>15,37</sup>

### Clinical Picture

The onset, signs, symptoms, and course of allergic purpura are quite variable. Headache, anorexia, fever, or abdominal pain may usher in an attack, or pain in and around the joints may be the first complaint. Purpura may be a relatively minor component of the clinical picture, and in some attacks may even be absent. Intermittent fever of moderate degree is common.

**SKIN LESIONS.** Purpura usually is associated with one or more of the common cutaneous manifestations of allergy, eg, urticaria, erythema (Table 36-2 and Fig. 36-1), and the resulting skin lesions may "run the gamut of the skin atlas."<sup>43</sup> The lesions usually are located on the proximal portions of the extremities, particularly the legs and on the buttocks, often are symmetric in distribution, and may appear and regress in crops. In contrast to most other purpuric lesions, they may be accompanied by itching or paresthesias.

Osler,<sup>43</sup> who described the lesions in great detail, pointed out that there may be four major types of lesions: (1) purpura which may be simple but more often is accompanied by swelling; blebs may be found which have the appearance of herpes on a purpuric or hyperemic base or there may be bullae or even pemphigoid lesions (Fig. 36-2); (2) urticarial wheals or angioneurotic edema; (3) diffuse erythema, with or without swelling; (4) necrotic areas which may be followed by the formation of ulcers.<sup>31</sup>

Gairdner<sup>24</sup> described the evolution of skin lesions which he regarded as very characteristic. Small, discrete urticae first appear upon the extensor surfaces of the upper and lower limbs. Within a few hours these begin to change to pink maculopapules, becoming less raised and darker in color. Dusky red macules which do not fade on pressure are found the next day and these may coalesce

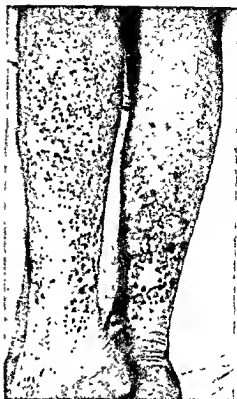


Fig. 36-1 Hemorrhagic and erythematous lesions in a patient with Henoch-Schönlein purpura

to form larger patches (Fig. 36-3). As regression takes place, the red color takes on a purple hue before fading to brown. Frankly hemorrhagic lesions are uncommon.

**GASTROINTESTINAL TRACT.** Serohemorrhagic effusions into the bowel wall may lead to abdominal colic, nausea, and vomiting in approximately 50% of cases. When such abdominal symptoms predominate, the disorder is often referred to as Henoch's purpura.<sup>30</sup> As stated earlier, these symptoms may develop before the purpuric eruption appears; needless operations have been performed as a result.<sup>1,7</sup> Colic is the most common symptom. The pain may radiate to all parts of the abdomen, but usually is midabdominal in location. In Osler's experience<sup>43</sup> the right lower quadrant was spared, and colic occurred most often at night. Tenderness is often present, but muscular rigidity is absent. Diarrhea is uncommon, but mucoid or bloody stools may be passed. Gross melena,

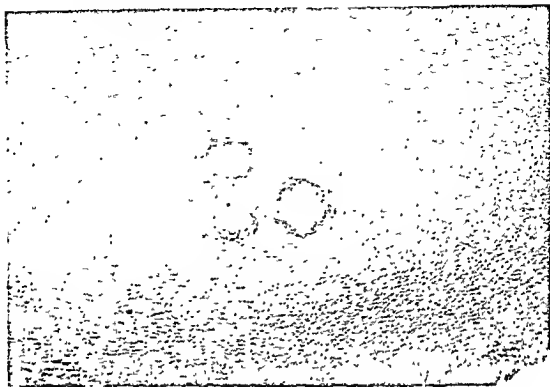


Fig 36-2 Bullous lesions in a patient with allergic purpura (Courtesy of Dr Peyton Weary)

hematochezia, and intestinal perforation<sup>44</sup> are rare complications.<sup>27</sup> Tenesmus may be noted and constipation may be so stubborn as to suggest obstruction. Intussusception has been observed<sup>93</sup> and is more common in children than in older subjects. Protein-losing enteropathy was reported in five cases.<sup>32</sup> Small isolated red spots may be seen in the buccal mucosa. Gastrointestinal roentgenograms reveal various nonspecific abnormalities.<sup>29,43,49</sup>

**MUSCULOSKELETAL SYSTEM.** The term Schönlein's purpura refers to allergic purpura in which joint pain and tenderness are prominent.<sup>51</sup> The knees, ankles, and wrists are most commonly involved.<sup>49</sup> These symptoms may precede the onset of abdominal pain or purpura.<sup>51</sup> The pain is rarely as intense as in acute rheumatic fever, and is not strikingly influenced by salicylates. Periarthicular swelling is common, but hemarthrosis does not occur. Edema may be present, particularly of the dorsum of the hands and feet.

**GENITOURINARY TRACT.** Renal involvement can be demonstrated at some time during the course of the disease in 22 to 60% of the patients.<sup>25,47,54-58</sup> In the majority, this is asymptomatic and completely reversible, but in 5 to 10% the picture of chronic or subacute glomerulonephritis develops.

In an occasional patient<sup>43</sup> the picture of fulminating acute glomerulonephritis with hypertension, uremia, and early death is seen.<sup>25</sup> Such a fatal course is more common in adults than in children.<sup>8</sup> Rarely, the nephrotic syndrome develops.<sup>25</sup> Whether or not these cases represent a fundamentally different disorder remains uncertain.<sup>16</sup>

**MISCELLANEOUS.** Wheezing and dyspnea have been described<sup>43</sup> but are unusual. Acute respiratory obstruction due to edema of the glottis is a serious complication but fortunately is rare. Myocardial necrosis,<sup>36</sup> hepatomegaly,<sup>3</sup> and hemorrhage into the testis<sup>3</sup> have been reported. Involvement of the nervous system<sup>33</sup> and special sense organs may



Fig 36-3. Erythematous macular lesions in a patient with allergic purpura (Courtesy of Dr Peyton Weary)

lead to such diverse symptoms as transient attacks of paresis; epileptiform convulsions; facial nerve palsy<sup>34</sup>; hemorrhages into the eyelids, conjunctiva, or retina; optic atrophy; iritis; and ophthalmitis.<sup>9,28</sup>

### Laboratory Findings

Examination of the blood may reveal modest neutrophilia or eosinophilia. Hemorrhage is rarely sufficiently marked to produce anemia. The reaction to the tourniquet test may be positive,<sup>45</sup> but the results of other tests of hemostasis and blood coagulation, including the platelet count and the bleeding time usually are normal.

Gross hematuria is uncommon. Microscopic hematuria, red cell casts, proteinuria, and mild azotemia are common but often

transient findings. Examination of the stools may reveal blood. The ASO titer usually is normal.<sup>55,142</sup> Elevated levels of IgA globulins have been demonstrated in approximately 50% of the patients.<sup>53</sup>

### Differential Diagnosis

The skin lesions of allergic purpura often are characteristic. This disorder should be suspected whenever erythematous or urticarial lesions are associated with purpura. A history of previous attacks of joint symptoms when the main complaint is referred to the abdomen, and vice versa, also is suggestive. Serious diagnostic difficulties usually arise when purpura is not obvious or is absent altogether. In such cases, the symptoms may suggest a variety of conditions. For example, bouts of abdominal pain accompanied by fever, leukocytosis, or melena cannot be readily distinguished from symptoms due to acute abdominal conditions which call for surgical intervention. When renal involvement is prominent, allergic purpura is readily mistaken for acute post-streptococcal glomerulonephritis. The serum complement or  $\beta_2$  globulin levels usually are low in glomerulonephritis,<sup>5,56</sup> but are normal in allergic purpura.<sup>55,112</sup> When joint symptoms predominate, a normal ASO titer is helpful in excluding the possibility of acute rheumatic fever.<sup>4,7</sup>

Skin and renal biopsies may be helpful in an occasional case, but the essentially non-specific findings discussed below cannot be regarded as diagnostic.<sup>142</sup> It is as yet uncertain whether various subtle ultrastructural features of the renal lesion as revealed by electron microscopy are more specific.<sup>1,12</sup>

### Course; Prognosis

Allergic purpura usually is a self-limited disorder and individual attacks last from one to six weeks, during which the symptoms may wax and wane in intensity. Recurrences, after an interval usually of days but sometimes of weeks or months, are very common, and in one case were observed over a period

of 17 years.<sup>18</sup> The recurrent clinical manifestations do not necessarily resemble those of the initial episode.

The immediate prognosis usually is favorable unless such complications as intussusception, hemorrhage into the nervous system, or edema of the epiglottis occur. In 50% of 49 patients having initial renal involvement, the urine was found to be normal three months later. However, in a significant number of patients, renal involvement may become chronic. Recovery was not observed in any patient in whom abnormal urinary findings were present over a period of two years or more.<sup>34</sup> It has been suggested that renal sequelae of allergic purpura are one of the causes of "chronic renal disease of unknown etiology" in adults.<sup>35</sup>

### Treatment

Because of the tendency of allergic purpura to remit and recur spontaneously, the efficacy of various therapeutic measures is difficult to evaluate. In the majority of cases, only symptomatic therapy is required. If it can be discovered, the inciting agent should be removed or avoided. Elimination diets may be tried. Results of treatment with antihistamines and adrenocorticosteroids, with few exceptions,<sup>11</sup> have usually been equivocal or disappointing. Corticosteroids, eg, prednisone, 2 mg/kg/day up to a maximum of 50 mg daily,<sup>36</sup> may provide symptomatic relief of joint and abdominal pain in some patients.<sup>11</sup> Immunosuppressive drugs have produced encouraging results in a few patients.<sup>27-30</sup> In the hope of reducing the incidence of chronic nephritis, bed rest has been recommended.<sup>1</sup>

### Pathology<sup>10,142</sup>

The skin reveals perivascular infiltration of the dermis and swelling and degeneration of endothelial cells. Numerous leukocyte platelet thrombi are present in small dermal vessels. In severe cases a necrotizing arteriolitis has been observed.<sup>21,24</sup> Kidney biopsies<sup>28a</sup>

obtained during the acute phase of the disease reveal focal fibrinoid deposits and endothelial proliferation within scattered glomeruli. Specimens taken during recovery show focal glomerular scars.<sup>38</sup> This contrasts with the diffuse and uniform changes observed in acute and subacute glomerulonephritis. More diffuse glomerular lesions have been found in some patients with chronic disease.<sup>30</sup> The lesion most commonly seen in the gastrointestinal tract at operation is extravasation of blood or serosanguineous fluid into the wall of the small intestine<sup>4</sup>; edematous, scarlet-colored segmental lesions also have been described.<sup>7</sup>

### Drug-Induced Vascular Purpura

A multiplicity of drugs and chemicals (Table 36-1) may produce striking generalized purpuric eruptions. Although often quite alarming to the patient, such drug-induced vascular purpura usually subsides promptly when use of the drug is discontinued. The purpura is not associated with other bleeding manifestations, and is thus of little clinical consequence.<sup>1</sup> Owing to the benign nature of the disorder, it is probable that unreported cases of vascular purpura have developed following the use of many drugs in addition to those listed in Table 36-1.

The pathophysiology of these lesions is poorly understood. Purpura develops in only an occasional patient who has taken these drugs and apparently is the result of individual idiosyncrasy. An autoimmune basis has been suggested, but direct evidence to support this hypothesis is lacking. Serologic studies have failed to demonstrate humoral antibodies.

The hemorrhagic skin infarcts which may develop following the administration of coumarin anticoagulants (page 1245) appear to represent an unusual form of drug-induced vascular purpura.

### Purpura Fulminans

The term "purpura fulminans" was applied by Henoch to a unique disorder characterized

by sudden onset, fever, prostration, symmetric circumscribed ecchymoses and infarcts of the skin, and frequently by gangrene of the extremities (Table 36-2). The major initiating factor in this disorder appears to be diffuse vascular injury, but in many well-studied cases there is clear evidence for diffuse intravascular coagulation. This disorder is discussed further on page 1219.

The term "purpura fulminans" also has been used in a less restricted sense to apply to any severe purpura of rapid onset, eg,

meningococcemia, particularly when associated with the Waterhouse-Friderichsen syndrome.

### Purpura Associated with Infections

A wide variety of infections may produce purpura by means of vascular damage (Table 36-1 and Fig. 36-4). This may result from direct endothelial injury by the infectious agent, eg, rickettsia, some viruses, meningo-



Fig 36-4. Purpuric lesions in a patient with scarlet fever. There was no thrombocytopenia (From Fox and Enzer,<sup>23</sup> courtesy of the authors and the American Journal of Medical Science)

cocci; in other cases, autoimmune processes, bacterial products, or toxins may be responsible.<sup>125</sup> In bacterial endocarditis, the purpura is due to embolic occlusion of the microvasculature, and white-centered hemorrhagic lesions may be seen. Purpura associated with meningococemia also may be due, in part, to emboli. In many cases, thrombocytopenia (page 1101) and diffuse intravascular coagulation (Chapter 38) contribute to the purpura associated with infectious diseases.

## Structural Malformations of Vessels and Perivascular Tissues

### Hereditary Hemorrhagic Telangiectasia

In hereditary hemorrhagic telangiectasia, a vascular malformation involves vessels throughout the body which are dilated, tortuous, and disorganized. Widespread telangiectatic lesions of the skin and mucous membranes are the principal manifestation of the disorder. The walls of affected vessels are markedly thinned, vascular support is poor and vascular contractility is diminished. As a result, bleeding may follow trivial trauma or arise spontaneously. Hereditary telangiectasia was first described by Sutton<sup>65</sup> in 1864 and received the attention of Rendu<sup>66</sup> in 1896, Osler<sup>67</sup> in 1901, and Weber<sup>68</sup> in 1907, by whose names the condition is sometimes known.

### Etiology, Genetics

The vascular abnormality is inherited as an autosomal dominant trait of high penetrance.<sup>67</sup> Some individuals rarely or never suffer from hemorrhage and this may explain the apparent skipping of the members of a whole generation. Painsstaking examination usually reveals signs of the disease in one of the parents. Rarely, a new mutation occurs or the lesions are all internal. The manifestations in the offspring of two affected parents were severe and extensive from birth, and death occurred at 11 weeks of age.<sup>64</sup>

The condition probably is not as rare as was once thought. In Utah, the polygamy of the pioneers led to the wide transmission of the trait, as the family tree shown in Figure 36-5 testifies. Affected kindreds have been chiefly of Anglo-German, Latin, Scandinavian, or Jewish stock. There are three reports of the disorder in the Negro.<sup>69</sup>

### Clinical Picture

The lesions range in size from pinpoint to about 3 mm in diameter. They are bright red or purple, and are most commonly found on the face, lips (Fig. 36-6), tongue, ears, conjunctivae, and the palmar and plantar surfaces<sup>66</sup> (Fig. 36-7). They usually are flat. Characteristically, they blanch on pressure (Table 36-3). To observe this, pressure may be applied by means of a glass slide through which the color of the lesion may be observed. Purpura and ecchymoses are not seen, and, unlike purpuric lesions, telangiectases are permanent. The telangiectases may form nodular vascular tumors the size of a split pea (Fig. 36-8), and sometimes they are spider-like, particularly in elderly patients. The last-named lesions, however, are not characteristic of the disorder.

The most common symptoms are those of hemorrhage and anemia. In general, telangiectases of the skin are less likely to bleed than are those of the mucous membranes. Epistaxis is especially common, but bleeding may come from telangiectases in any location such as the tongue or mucous membranes of the mouth, or the gastrointestinal,<sup>102</sup> respiratory, or genitourinary tracts.<sup>76</sup> Hemorrhage even into the brain and retina<sup>71</sup> has been attributed to the presence of telangiectases, but telangiectatic lesions have not been demonstrated in all instances. Bleeding from wounds or following surgical procedures is uncommon. Pallor may or may not be present. Splenomegaly and hepatomegaly have been found in a few cases, and are most common in older patients.<sup>75,91</sup>

The telangiectases may be found in childhood but they increase in number with advancing age. Bleeding may not commence

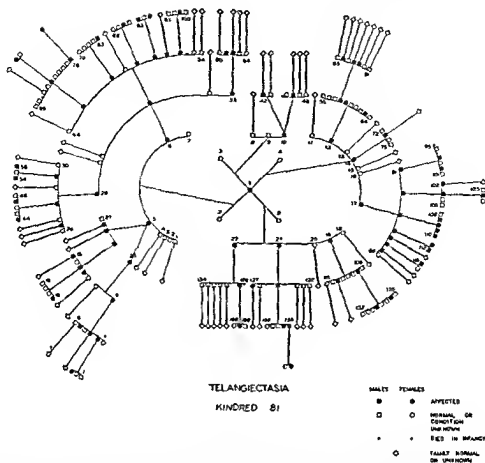


Fig 36-5. Family tree showing six generations of a family of 'bleeders' whose progenitor had four wives<sup>72</sup> Those persons who were examined were found to have hereditary hemorrhagic telangiectasia (Courtesy of Dr F E Stephens)

Fig 36-6 Typical telangiectases on the lips of a patient with hereditary hemorrhagic telangiectasia



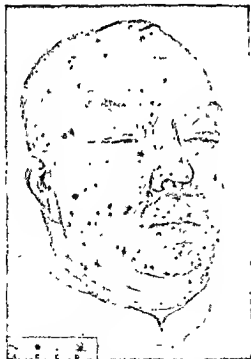
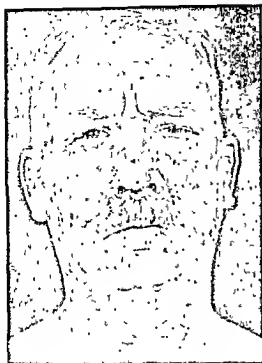


Fig. 36.8 Photograph and drawing of the head of the patient whose hands and feet are shown in Figure 36.7. Four types of vascular lesions were present. Inset B is the typical telangiectasis. C is a spider angioma.

reactions to the tourniquet test<sup>101</sup> and prolongation of the bleeding time have been recorded. In an occasional patient, diminished *in vivo* platelet adhesiveness<sup>81</sup> and other findings suggestive of von Willebrand's disease<sup>86</sup> (page 1179) have been encountered. These findings have led to speculation regarding a nosologic relationship between the two disorders.<sup>86</sup>

### Differential Diagnosis

Diagnosis of hereditary hemorrhagic telangiectasia is seldom difficult because the triad of habitual hemorrhage, multiple telangiectases, and familial occurrence is so characteristic. The telangiectases must be differentiated from purpuric lesions, spider telangiectases of liver disease, cherry angiomas, and the various venous abnormalities which are frequently seen in elderly persons, eg, venous lakes, venous stars, caviar lesions on the under surface of the tongue, and senile telangiectases of the scrotum (angiokeratoma of Fordyce) (Table 36-3). When externally visible lesions are lacking or are overlooked, perplexing diagnostic problems may result. In such cases, erroneous diagnoses such as pernicious or other forms of anemia, peptic ulcer, or Banti's syndrome have been made. This disorder should always be considered in the differential diagnosis of recurrent or intractable gastrointestinal bleeding of obscure etiology.<sup>102</sup> In such cases, visceral angiography may be of great help in establishing the diagnosis.<sup>79</sup>

Pulmonary arteriovenous fistulas are best demonstrated by fluoroscopy or angiocardiology. Ordinary roentgenograms may not always reveal the lesions.<sup>70</sup>

The telangiectatic skin lesions of scleroderma, and those of a related syndrome characterized by Raynaud's phenomenon, sclerodactyly, and subcutaneous calcinosis, are virtually identical in appearance, but are most common on the hands, seldom bleed, and are rarely found in the gut.<sup>65,99,103</sup> The skin lesions of the rare angiokeratoma corporis diffusum universale (CDU) (Fabry)<sup>119</sup> may be confused with those of hereditary telangi-



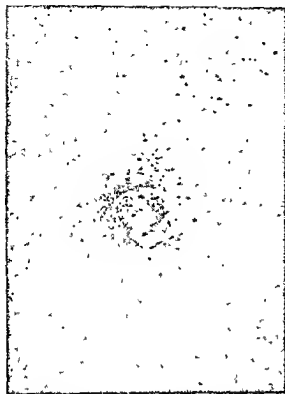


Fig. 36-9. Lesions resembling purpura in a patient with *angiokeratoma corporis diffusum universale*

ectasia as well as with petechiae (Table 36-3 and Fig. 36-9). This disorder (page 1340) is an inherited abnormality of glycolipid metabolism resulting from deficiency of a ceramide-trihexoside-cleaving enzyme. It is characterized by widespread involvement of the media of blood vessels including the renal and pulmonary vasculature.<sup>134</sup>

### Treatment

Only symptomatic and supportive therapeutic measures are available. Oxycel, Gel-foam, or similar topical hemostatic agents usually are effective in controlling hemorrhage from accessible sites, and they have the added advantage that they do not need to be removed after the bleeding has stopped. Nasal tamponade may be necessary in the treatment of epistaxis. A useful device consists of a finger cot placed over the end of a small catheter and tied snugly with fine thread. This is lubricated, inserted well back in the nostril, and inflated. Firm uniform

pressure is thus applied to the entire interior of the nasal fossa. After the bleeding has stopped, the cot is slowly deflated and withdrawn or allowed to drop out of the nostril.<sup>74</sup>

Although it is possible to destroy the primary lesions by *cautery* with escharotics such as silver nitrate or by means of electrocoagulation,<sup>74</sup> such therapy is usually futile since satellite lesions soon form nearby. Electrocoagulation has proved more effective in destroying lesions about the lips, the oral cavity, and on the cutaneous surface of the body than in the nasal fossae. Septal dermoplasty was reported as being effective in permanently controlling epistaxis.<sup>59</sup> Radium, although successful immediately, eventually produces atrophy and dryness of the nasal mucosa and may be followed by perforation of the septum.

The systemic administration of estrogen alone<sup>81,83</sup> (0.25 to 1.0 mg ethinyl estradiol per day) or in combination with a progestational steroid<sup>73</sup> is claimed to be effective in reducing the frequency and severity of epistaxis. These hormones induce squamous metaplasia of the nasal mucosa, which presumably protects the vascular lesions from trauma.<sup>128</sup> In males, feminizing side effects may be minimized by the concomitant administration of methyl testosterone (2.5 to 5.0 mg daily).

*Arteriovenous fistulas*, if symptomatic, should be treated surgically. Unfortunately, the lesions often are more diffuse than suspected preoperatively and recurrences are common.

Iron deficiency anemia should be treated with sufficient iron to replenish tissue stores (Chapter 17). The intravenous administration of massive amounts of iron dextran compounds may be necessary in the face of continued blood loss.<sup>69</sup>

### Pathology

The walls of the affected vessels are extremely thin, consisting merely of a layer of endothelium.<sup>80</sup> The veins are involved primarily and arterial involvement is inconspicuous. The telangiectases have been found at

autopsy<sup>85,91</sup> in all major organ systems and in each the venous defect was diffusely distributed.<sup>64</sup> The large arteriovenous aneurysms found clinically in some patients may be only the larger counterparts of widespread telangiectases in the pulmonary tissue.<sup>100</sup>

### Hereditary Disorders of Connective Tissue

Abnormal bleeding is a common and clinically significant complication of the various hereditary disorders of connective tissue. In the Ehlers-Danlos syndrome and in osteogenesis imperfecta, qualitative and quantitative abnormalities of collagen, and possibly of elastin as well, presumably underlie the vascular abnormality. These result in vascular fragility and bleeding because of deficient extravascular and perivascular tissue support. Large ecchymoses and hematomas are common, but virtually all bleeding manifestations have been described.<sup>148</sup> Various abnormalities of platelet function, and rarely of blood coagulation, may contribute to the bleeding in an occasional patient. In a related disorder (hydroxylysine deficient collagen disease), easy bruising was the only hemorrhagic manifestation.<sup>116a</sup> In pseudoxanthoma elasticum, a wide variety of hemorrhagic manifestations is common, and subarachnoid and gastrointestinal bleeding are the most common causes of death.<sup>36</sup> There is some evidence that the fundamental vascular defect in this disorder is a structural abnormality of elastic fibers in small arteries.

Diagnosis is made on the basis of the associated clinical findings, which usually are characteristic. There is no known therapy.

### Acquired Disorders of Connective Tissue

#### Scurvy

Scurvy may be associated with serious bleeding manifestations, including persistent gingival bleeding and hemorrhage into the subcutaneous tissues and muscles.<sup>113</sup> Petechiae often develop in a characteristic "sad-



Fig. 36-10 Perifollicular petechiae in scurvy (From Weary,<sup>141</sup> courtesy of the author and Archives of Dermatology.)

dle" distribution (medial surface of the thighs, buttocks) and are most conspicuous around the hair follicles<sup>36,145</sup> (Table 36-2 and Fig. 36-10). Subperiosteal hemorrhages are characteristic of infantile scurvy but are very rare in adults. Bleeding is attributed to a defect in the endothelial lining and perivascular supporting tissues of small vessels. This is due to deficient synthesis of collagen and intercellular cement substance, but the biochemical basis for this defect remains obscure despite intensive study. The vascular abnormality may be complicated by disordered platelet function in some cases (page 1132). An abnormality in the contact phase of blood coagulation has been described in scorbutic guinea pigs.<sup>121</sup> Evidence that bleeding in scurvy is due to deficiency of a poorly characterized flavone ("vitamin P") is unconvincing.<sup>139</sup>



Fig 36-11 Senile purpura

A positive reaction to the tourniquet test is commonly seen and the bleeding time is occasionally prolonged. With these exceptions, the results of laboratory studies of hemostasis and blood coagulation are normal. Mild thrombocytopenia and other evidence of associated folic acid deficiency (Chapter 14) are not uncommon.

The oral administration of ascorbic acid (in adults, 1 g per day in divided doses; in infants, 50 mg) rapidly abolishes all hemorrhagic manifestations. Intravenous therapy offers no advantages, since the major proportion of the vitamin administered by this route is lost in the urine.

### Senile Purpura

Senile purpura is a chronic disorder of the elderly characterized by relatively distinctive red to purple ecchymotic spots on the ex-

tensor surfaces and radial borders of the forearm (Fig. 36-11), and the backs of the hands and neck.<sup>160</sup> The lesions range up to 4 cm in diameter. They usually arise spontaneously, but may result from very trivial pressure, eg, eyeglasses on the bridge of the nose (Table 36-2). The basic defect is one of degeneration and loss of dermal collagen, elastin, and subcutaneous fat. The distribution of the lesions of senile purpura corresponds in general to areas subjected to life-long exposure to actinic irradiation.<sup>142</sup> There is good evidence that the purpura is the result mainly of shearing injury of small dermal vessels which results from the hypermobility of the skin over underlying tissues.<sup>154</sup> Senile purpura may persist for weeks, seldom reveals the sequential pigmentary changes normally seen with other ecchymoses, and often leaves a residual brownish pigmentation. The indolent nature of the lesions has been attributed to inadequate mobilization of extravasated hemoglobin due to deficient macrophage function.<sup>154</sup> Liver disease was commonly associated in one series.<sup>118</sup> There is no therapy of proven value, and reports of the efficacy of ascorbic acid have not been confirmed.<sup>108</sup> Similar skin lesions are seen in a variety of debilitating disorders (*purpura "cachectica"*).

Vascular fragility manifested by "easy bruising" from trivial trauma is a predictable pharmacologic effect of the prolonged administration of moderate to large doses of corticosteroids. In terms of appearance, histopathology, and indolent course, the lesions resemble those of senile purpura. Similar abnormalities presumably are responsible for the purpura which may be seen in Cushing's disease.

## Miscellaneous Vascular Purpuras

### Autoerythrocyte Sensitization and Related Disorders

Autoerythrocyte sensitization is an uncommon disorder characterized by spontaneous

painful ecchymoses. Often heralded by prodromal stinging or burning, the lesions usually are surrounded by erythema and edema and may enlarge progressively (Table 36-2). They are commonly associated with headache, nausea, and vomiting, and occasionally with intracranial, genitourinary, and gastrointestinal bleeding as well.<sup>150</sup> In the majority of patients, similar ecchymotic lesions can be reproduced by the intradermal injection of autologous whole blood,<sup>123</sup> washed red cells, red cell stroma,<sup>4</sup> or even phosphatidylserine derived from the red cell membrane.<sup>126</sup> This relatively specific laboratory finding has led to the hypothesis that the purpura is the result of autosensitization to some component of the red cell membrane. In most of these patients, the "sensitizing incident" was unclear,<sup>150</sup> but in many there was a past history of severe trauma with bruising.

The disorder has occurred almost exclusively in women of middle age, and several observers have suspected that emotional factors may play an important pathogenetic role.<sup>106, 149, 150</sup> Psychiatric study revealed the propensity of such patients to express emotional problems in a physical form through both hysterical mechanisms and psychophysiologic reaction. Others manifested a prominent element of masochism in their character. Purpuric bouts were found to occur at times of emotional stress. Psychologic testing has revealed a remarkably uniform personality pattern characterized by difficulty in handling aggressive feelings and by other features which favor the view that this condition may be psychosomatic in nature.<sup>111</sup> In four patients,<sup>106</sup> purpura apparently was induced by hypnotic suggestion. This remarkable and important observation should be confirmed.

Autoerythrocyte sensitization may, thus, be a psychopathologic entity and certainly deserves further study from this point of view. The relation of this form of purpura to the hemorrhagic stigmata of religious connotation, which from time to time have received wide attention, has been considered.<sup>151</sup> Like the latter, in some instances of autoerythro-

cyte sensitization the purpura was shown to be factitious.<sup>114, 137</sup>

The clinical features of DNA auto-sensitivity<sup>112, 140, 152</sup> resemble those of autoerythrocyte sensitization. The acute and painful ecchymoses are confined to the extremities and can be produced by the intradermal injection of a solution of the patient's leukocytes or a solution of deoxyribonucleic acid (DNA) into the skin of the extremities. *In vitro* incubation of these substances with deoxyribonuclease, chloroquine, or primaquine abolishes this effect. Similar injections of ribonucleic acid fail to produce hemorrhages. Skin from an extremity which has been grafted to the trunk fails to react, but skin from the trunk will react if grafted to an extremity. The condition appears to represent localized hypersensitivity to DNA. In yet another similar disorder,<sup>133</sup> purpura could be reproduced by the intradermal injection of autologous hemoglobin.

These disorders may persist for years, often with remissions and exacerbations, and, in contrast to most other forms of vascular purpura, they may be severely debilitating. There is no treatment of proven value. Corticosteroids and antihistamines have usually been ineffective, and psychotherapy is seldom of permanent benefit. In DNA auto-sensitivity, treatment with chloroquine, primaquine, or hydroxychloroquine produces a dramatic clinical response, but relapse follows cessation of drug therapy.

#### Purpura in Association with the Paraproteinemias

In purpura associated with the paraproteinemias (Chapter 53), bleeding is an important clinical problem, but the hemostatic defect is complex, and the contributory roles of platelet dysfunction (page 1130), coagulation abnormalities (page 1211), and thrombocytopenia are difficult to assess in the individual case. In many patients, vascular abnormalities appear to predominate, and have been attributed to a direct or indirect

effect of the abnormal protein, eg, hyperviscosity and red cell "sludging" with thrombosis, a direct toxic effect of paraproteins, and even an autoimmune process.<sup>112</sup> There is no convincing evidence for any of these hypotheses.

Primary hyperglobulinemic purpura<sup>125, 159, 161, 163</sup> is a disorder most commonly seen in women which is characterized by hyperglobulinemia of the polyclonal type, and recurrent acute episodes of purpura, especially after unusual exertion, prolonged standing or excessive pressure from garments.<sup>108a</sup> Lesions are most common on the lower extremities, frequently are associated with premonitory itching, stinging, and erythema, and commonly result in progressive deposition of pigment.<sup>124</sup> The disorder thus resembles Schamberg's progressive pigmentary purpura.<sup>112</sup> Immunoelectrophoretic studies have demonstrated large amounts of anti-IgG globulins and antigen-antibody complexes formed therefrom.<sup>111</sup> Prolonged follow-up of patients with "benign" primary hyperglobulinemic purpura has revealed that the disorder frequently terminates in Sjogren's syndrome<sup>109, 124, 162</sup> and various other disorders.<sup>108a, 145</sup>

Secondary hyperglobulinemic purpura has been described in virtually every disorder characterized by hyperglobulinemia, but the specificity of such a diagnostic entity is questionable.<sup>159</sup>

Cryoglobulinemia of both the primary and secondary types (page 1640) gives rise to a relatively distinctive cold sensitivity syndrome, of which purpura is an important feature.<sup>127, 162</sup> Bleeding has been attributed to intravascular precipitation of cryoglobulins with resulting vascular damage,<sup>127</sup> and, in some cases, can be produced by placing an ice cube against the skin. Purpura is most common on the extremities, nose, ears, and face; bullae, chronic ulcerations, and cold urticaria are commonly associated. Cryofibrinogenemia produces a similar clinical picture,<sup>164</sup> presumably as the result of a related phenomenon, ie, the formation of cryoprecipitable complexes between fibrinogen,

plasma globulins, and various fibrinogen degradation products (page 438).

In Waldenström's macroglobulinemia, mucosal bleeding is more prominent than cutaneous hemorrhage, and epistaxis, gingival bleeding, and vaginal bleeding are common.<sup>143</sup> IgM globulins may also behave as cryoglobulins.

There are no specific measures for the treatment of bleeding in any of these conditions. Therapy of the underlying disorders is discussed in Chapters 52 and 53.

### Purpura Simplex and Related Disorders

The term "purpura simplex" usually refers to mild purpuric skin manifestations in otherwise healthy persons. The disorder appears to be particularly common in women ("devil's pinches") and often involves only isolated small ecchymoses on the legs. Exacerbations of such purpura during the menstrual period are not uncommon. In a hereditary form, spontaneous ecchymoses accompanied by positive reactions to the tourniquet test were observed in 88 members of 27 families.<sup>115</sup> All but four of those affected were females. Rheumatoid arthritis and rheumatic fever frequently were associated. Other cases have been reported<sup>120, 157</sup> which may or may not belong in this group. It is likely that, in some cases previously classified as "purpura simplex," bruising was the result of hereditary or drug-induced abnormalities of the platelet release reaction (Chapter 35). The disorder is usually of cosmetic significance only, and therapy is not required.

Violent muscular contractions, such as occur in whooping cough or convulsions, produce marked increases in intracapillary pressure which may cause vascular rupture and subcutaneous extravasations of blood. Such "mechanical purpura" usually involves the head, neck, and upper extremities. The term "orthostatic purpura" describes purpura which develops for the same reasons in the lower extremities in some persons on prolonged standing. Neither term is particularly useful, since it should be recognized that

purpura of any type tends to develop in areas of increased venous or capillary pressure. Although both "mechanical" and "orthostatic" purpuras may rarely be seen in normal persons, they are much more likely to be the result of a definable abnormality, e.g., thrombocytopenia.

Self-inflicted or factitious purpura is relatively common and on occasion may lead to serious diagnostic problems. Very bizarre purpuric lesions may result from self-flagellation with various objects, pinching, or sucking of the skin. The disorder is most common in women, and neurotic or psychotic symptoms usually are prominent. Factitious purpura must be distinguished from autoerythrocyte sensitization and the bleeding which results from self-administration of anticoagulant drugs (page 1245).

#### Purpura Associated with Skin Diseases and Other Conditions

In certain skin diseases<sup>142-144</sup> a purpuric eruption unaccompanied by thrombocytopenia may be encountered. These include annular telangiectatic purpura (Majocchi's disease), Schamberg's disease, pigmented purpuric lichenoid dermatitis, and angioroma serpiginosum. A number of chronic diseases may occasionally be associated with vascular purpura. The purpura described in some patients with acute glomerulonephritis and rheumatic fever<sup>131</sup> is similar to that seen in patients with allergic purpura. Purpura may result from diffuse vascular infiltration in generalized amyloidosis,<sup>105a,147,148</sup> polycythemia vera, myxedema, rheumatoid arthritis, and in as many as 15% of patients with hemochromatosis.<sup>158</sup> The multiple hemorrhagic lesions in the serous membranes, lungs, and other viscera following poisoning by viperidae and crotaline snake venoms are due in part to direct injury of the endothelial lining of capillaries and small veins.<sup>132</sup> Blood-borne tumor emboli are a rare cause of extensive purpura.<sup>156</sup> The purpuric manifestations of hepatic disease (page 1205) and azotemia (page 1129) are discussed in other sections.

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## *The Hereditary Coagulation Disorders*

### **Nomenclature**

#### **Principles of Pathophysiology**

#### **Hemophilia A (Factor VIII Deficiency)**

#### **Hemophilia B (Factor IX Deficiency)**

#### **Factor XI Deficiency**

#### **Disorders of Fibrinogen**

##### **Afibrinogenemia**

##### **Hypofibrinogenemia**

##### **The Dysfibrinogenemias**

##### **Factor XIII Deficiency**

#### **Deficiency of Prothrombin**

#### **Factor V Deficiency**

#### **Factor VII Deficiency**

#### **Factor X Deficiency**

#### **Factor XII Deficiency**

#### **Von Willebrand's Disease**

#### **Miscellaneous**

##### **In Lower Animals**

### **Treatment**

#### **Replacement Therapy**

#### **Special Aspects**

far greater scientific interest than their statistical frequency would suggest is evident from the number of books,<sup>37,40,41,163,213</sup> monographs,<sup>68,69,70,325</sup> and reviews<sup>39,304,324,326,375</sup> that deal with these experiments of nature.

The hereditary coagulation disorders produce quite similar signs and symptoms regardless of the particular factor that is lacking. Consequently, in the discussion to follow, only the clinical picture of hemophilia A, the most common variety, will be described in detail. Clinical features of the other forms that differ significantly from this prototype will be cited in the sections dealing with these very rare disorders.

The treatment of the hereditary coagulation disorders will be discussed in a single section (page 1183).

## **Nomenclature**

Many names have been proposed for the hereditary coagulation disorders, most of which incorporate the term "hemophilia," eg, pseudohemophilia, deuterohemophilia, parahemophilia, hemophiloid states A, B, C, and D.<sup>67</sup> Although the international Roman numeral designations (Table 10-I, page 410) represent a useful nomenclature for the coagulation factors, they do not provide descriptive names for conditions characterized by qualitative abnormalities of the various factors. The use of the term "hemophilia A" now appears to be preferable to factor VIII "deficiency," as will be evident from the dis-

**H**EREDITARY disorders of coagulation usually are the result of a deficiency or abnormality of a single plasma protein. As a consequence, they have provided a unique opportunity to study the phenomena of blood coagulation. The information that has resulted constitutes the cornerstone of our knowledge of this complicated and fascinating process. Hereditary coagulation abnormalities are the least common of the hemorrhagic disorders; the absolute incidence of the 30 or more disorders that comprise this group (Table 37-1) totals at most 1 in 10,000 to 15,000 persons<sup>190,217,369</sup> That they are of

Table 37-1. Hereditary Disorders of Coagulation

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<i>X-Linked Recessive Traits</i>
Hemophilia A
Hemophilia B ("Bm variant" <sup>50,179</sup> , Leyden variant <sup>407</sup> )
<i>Autosomal Recessive Traits</i>
Factor XI deficiency
Prothrombin deficiency
Factor V deficiency
Factor VII deficiency
Factor X deficiency (Prower variant <sup>104,392</sup> , Stuart variant <sup>104,180</sup> , Friuli variant <sup>104,142</sup> , others)
Afibrinogenemia
Hypofibrinogenemia
Factor XII deficiency
Factor XIII deficiency
<i>Autosomal Dominant Traits</i>
von Willebrand's disease
The dysfibrinogenemias (fibrinogens Amsterdam, <sup>198</sup> Baltimore, <sup>22</sup> Bethesda I, Bethesda II, <sup>155</sup> Cleveland I, <sup>129</sup> Cleveland II, <sup>328</sup> Detroit, <sup>242</sup> Giessen, <sup>328</sup> Iowa City, <sup>196a</sup> Leuven, <sup>407a</sup> Los Angeles, <sup>411</sup> Metz, <sup>373</sup> Montreal, <sup>231a</sup> Nancy, <sup>388</sup> Oklahoma, <sup>162</sup> Paris I, <sup>252</sup> Paris II, <sup>348</sup> Philadelphia, <sup>245a</sup> St. Louis, <sup>158</sup> Troyes, <sup>373</sup> Vancouver, <sup>345</sup> Wiesbaden, <sup>427</sup> Zurich I, <sup>413</sup> Zurich II, <sup>332</sup> others <sup>193,393</sup> )
<i>Combined Abnormalities</i>
Associated with hemophilia A (factor V deficiency <sup>145,352</sup> hemophilia B <sup>52,341</sup> factor XI deficiency, <sup>380</sup> factor VII deficiency <sup>333</sup> von Willebrand's disease <sup>337,338</sup> dysfibrinogenemia <sup>357</sup> )
Involving vitamin K-dependent factors (factors II, VII, IX, and X <sup>245</sup> factors VII and IX <sup>134,284,411</sup> factor X and VII <sup>200</sup> , others)
Miscellaneous <sup>55,68,119,269,274,283,342,353</sup>
<i>Miscellaneous</i>
Carr factor deficiency <sup>43</sup>
Fletcher factor (pre-kallikrein) deficiency <sup>1</sup>
Dynia abnormality <sup>293</sup>
<i>Hereditary Coagulation Disorders in Lower Animals<sup>111a</sup></i>
Dogs (hemophilias A and B <sup>11,71,76,263</sup> , factor VII deficiency <sup>264,279</sup> , factor X deficiency <sup>114</sup> , factor XI deficiency <sup>111a</sup> , von Willebrand's disease <sup>111</sup> , hypofibrinogenemia)
Goats (hypofibrinogenemia)
Horses (hemophilia A <sup>279</sup> )
Swine (von Willebrand's disease <sup>175</sup> )
Cows (factor XI deficiency <sup>217</sup> )
Mice (prothrombin complex factors <sup>250</sup> )

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cussion below, and the term "hemophilia B" is equally logical, since this disorder, also known as factor IX deficiency and Christmas disease, so closely resembles hemophilia A. The term "deficiency" will be retained with reference to the various other disorders, but it will be used in a functional sense, and should not be construed as having any pathophysiologic significance.

As with abnormal hemoglobins, qualitatively abnormal fibrinogens are now designated by the name of the city in which they were first discovered, eg, fibrinogen Paris.

Specific terms will also be used for variants of other disorders if such terms have become widely used, eg, factor X *Friuli*. Rapid changes can be expected in this field, and efforts directed toward standardizing this admittedly unsatisfactory nomenclature hopefully will be forthcoming.

## Principles of Pathophysiology

With the exception of fibrinogen and prothrombin, the coagulation factors are trace

proteins that have been difficult to study directly by chemical methods. Traditional laboratory measurements of their activity are "bioassays," which are inherently incapable of distinguishing between a *quantitative* abnormality, i.e., the absence of a requisite factor, and a *qualitative* abnormality, i.e., a non-functioning factor that is present in normal amounts. For many years it was generally assumed that the hereditary coagulation disorders were the result of a quantitative deficiency of trace plasma proteins. However, studies employing various immunologic techniques have now demonstrated that the absence of coagulant activity in the plasma of patients with these disorders may result from either deficient biosynthesis of a requisite protein, or defective biosynthesis, leading to the production of normal amounts of functionally inactive or functionally abnormal analogs<sup>326</sup> (Table 37-2).

The results of immunologic tests for the presence of coagulation factors usually are expressed as positive or negative for "cross-reacting material" (CRM). A positive test for CRM implies that a substance that is antigenically similar to the normal coagulation factor is present in the plasma. A coagulation disorder characterized by the presence of

such a substance often is termed a "CRM-positive" or "qualitative" disorder or variant; examples of such disorders are hemophilia A and factor XIII "deficiency." Some qualitatively abnormal coagulation factors produce abnormalities in coagulation that differ from those associated with a true quantitative deficiency of the factor. For want of a more specific term, such factors are termed *abnormal or aberrant factors*, in order to distinguish them from completely nonfunctional analogs. The most clearly defined disorders of this type are the dysfibrinogenemias, in which the abnormal fibrinogen is not totally nonfunctional, but may inhibit the function of normal fibrinogen. Other disorders characterized by "aberrant" coagulation factors include the Bm variant of hemophilia B, and some of the variants of factor X deficiency. Aberrant procoagulant proteins also may be synthesized in acquired "deficiencies" of the vitamin K-dependent factors (page 414).

A negative test for cross-reacting material indicates the absence of antigenically competent protein, and suggests that the disorder is due to deficient biosynthesis of the requisite factor. Such "quantitative" or "CRM-negative" disorders include afibrinogenemia and factor V deficiency. Presently available

Table 37-2. Pathophysiologic Mechanisms in the Hereditary Coagulation Disorders

Disorder	Defective Biosynthesis		
	Deficient Biosynthesis	of Functionally Inactive Factors	of Functionally Abnormal Factors*
Hemophilia A	+	+	—
Hemophilia B	+	+	+
von Willebrand's disease†	+	+	?
Afibrinogenemia	+	—	—
The dysfibrinogenemias	—	+	+
Hypoprothrombinemia	+	+	+
Factor V deficiency	+	—	—
Factor VII deficiency	+	+	—
Factor X deficiency	+	+	+
Factor XI deficiency	+	—	—
Factor XII deficiency	+	—	—
Factor XIII deficiency	?	+	—

\* Factors producing coagulation abnormalities that differ from those associated with simple deficiency of the factor

† With reference to factor VIII deficiency

techniques do not, however, exclude aberrations of sufficient magnitude to alter the antigenic determinants of the molecule, the presence of a precursor or subunit of the active coagulation factor, or the production of an analog that is catabolized at an abnormally rapid rate.<sup>326,328</sup>

## Hemophilia A

A severe and frequently fatal hemorrhagic diathesis that affected the male children of certain families was well recognized in antiquity. This is evident from the writings of Rabbi Simon ben Gamaliel (A.D. 2nd century) in the Talmud, and those of Maimonides, the Hebrew physician and philosopher, and Albucasis, the Arab (12th century).<sup>344</sup> The disease was clearly described by Otto<sup>217,296</sup> in 1803, and by Nasse who, in 1820, formulated the law of transmission of hemophilia.<sup>267</sup> The "bleeder's disease" was named hemophilia by Schönlein in 1839, and this term has since been used to refer to forms of the disorder that are inherited as X-linked recessive traits.<sup>77</sup> In 1893, Wright called attention to the prolonged coagulation time. Other early investigators in this field were Addis,<sup>5</sup> Sahli,<sup>346</sup> and Howell and Cekada.<sup>182</sup> Very complete monographs<sup>49,77</sup> cover the early literature.

### Pathophysiology

The hemostatic abnormality in hemophilia A (factor VIII deficiency, classical hemophilia) has at one time or another been attributed to a vascular defect, a platelet defect,<sup>126,259</sup> and abnormalities in the activity of tissue factor.<sup>235</sup> All of these hypotheses have been refuted. The apparent resistance of hemophilic platelets to aggregation and rupture<sup>182</sup> was found to be the result rather than the cause of the delayed coagulation.<sup>67,308,429</sup> The hypothesis that an excessive amount of a plasma inhibitor is responsible for the coagulation abnormalities in hemophilia was championed by Tocantins and his associates,<sup>394</sup> This hypothesis has gained few adherents,<sup>240,243</sup> and it is now generally ac-

cepted that the fundamental abnormality in hemophilia A is a deficiency or abnormality of a plasma protein.

Addis, in 1910,<sup>5</sup> demonstrated that a substance in normal plasma shortened the clotting time of hemophilic blood, thus suggesting that the disease is due to deficiency of a plasma protein. This substance (the anti-hemophilic factor, AHF, AHG, factor VIII) proved elusive and difficult to purify, and despite arduous efforts it is only within recent years that it has been studied in a semi-purified form (page 417). Such preparations of factor VIII are capable of correcting all coagulation abnormalities in the blood of hemophiliacs; they are equally effective *in vitro* and *in vivo*; their administration can prevent and arrest hemorrhage in patients with hemophilia A.

There is good evidence, however, that hemophilia A is not the result of deficiency of factor VIII; rather it is due to a *qualitative abnormality* of this protein. Thus, material which cross-reacts with antibodies to factor VIII was demonstrated in the plasma of each of 22 hemophilic patients from 21 different kindreds.<sup>432</sup> The amount of CRM, as quantified by immunoelectrophoresis, was the same in normal persons as in hemophiliacs. These results have been confirmed,<sup>31,383</sup> and normal or increased amounts of CRM have been demonstrated in the plasma of hemophiliacs by means of hemagglutination inhibition<sup>183,383</sup> and radioimmunoassay.<sup>184</sup> A quantitative variant of the disorder, in which CRM could not be demonstrated in the plasma, has been documented in approximately 5% of cases.<sup>31,84,384</sup> Available evidence thus suggests that a substance antigenically similar to factor VIII is present in normal amounts in the plasma of most patients with hemophilia A. This appears to be a nonfunctional form of factor VIII, which may be either a qualitatively defective form of this factor or a precursor (page 1180).<sup>326,328</sup>

### Genetics

Hemophilia A is the classic example of an X-linked recessive trait. The genetics of this

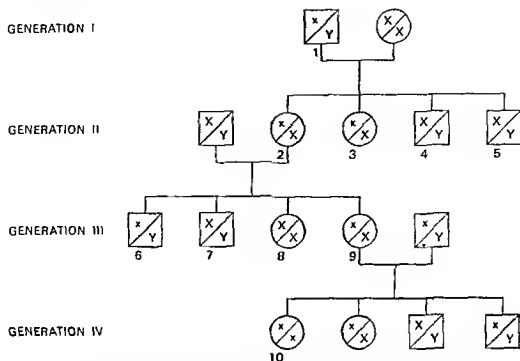


Fig 37-1 The inheritance of hemophilia A and hemophilia B. The pedigree is hypothetical. Key: normal X chromosome = X, abnormal X chromosome = x, male = square, female = circle, affected member = fully shaded square or circle, carrier = half shaded circle.

disorder have been intensively studied and frequently reviewed.<sup>49, 90, 150, 151, 194, 304</sup> In such a disorder, the defective gene is located in the X chromosome.<sup>52</sup> In males, who lack a normal allele, the defect is manifested by clinical hemophilia (Fig. 37-1, generation I, number 1). The affected male will not transmit the disorder to his sons (generation II, numbers 4 and 5) since his Y chromosome is normal. However, all of his daughters will be carriers of the trait since they inherit his X chromosome (generation II, numbers 2 and 3), but will be clinically unaffected due to the presence of a normal allele from the mother. The carrier female will transmit the disorder to one half of her sons (generation III, numbers 6 and 7) and the carrier state to one half of her daughters (generation III, numbers 8 and 9).

The severity of bleeding, ie, the *expressivity* of the genetic defect, varies from kindred to kindred in hemophilia A (Table 37-3). However, within a given kindred, the clinical

severity of the disorder is relatively constant, ie, relatives of severe hemophiliacs are likely to be severely affected.<sup>297</sup> These observations led to the suggestion that the degree of factor VIII deficiency depends on which of a series of abnormal alleles replaces the normal gene on the X chromosome.<sup>152, 310</sup> No conclusive evidence has yet been obtained that the factor VIII levels in normal subjects<sup>298, 305</sup> are genetically controlled.<sup>213</sup> Hemophilia A has been observed several times in *twins*.<sup>314</sup> Only in one somewhat equivocal instance has the disorder not appeared in both members of an identical pair.

It might be expected that, with random mating in a large population, a rare defect like hemophilia A would tend to die out after several generations, particularly since in the past the disorder was often fatal in childhood. That this has not occurred suggests that the mutation rate for the responsible gene may be unusually high.<sup>15, 100, 127, 309</sup> Consistent with this hypothesis is the fact that oo-ivi-

dence or history of abnormal bleeding is found in other members of the families of at least one third of all hemophiliacs.<sup>39</sup> The passage of the trait through a succession of carrier females or neonatal deaths may explain the negative family history in other instances. For practical purposes, therefore, a negative family history is of little value in excluding the possibility of hemophilia.

There is now good evidence that factor VIII production is regulated not only by the gene on the X chromosome, but also by one or more autosomal genes. This hypothesis has been established beyond doubt by the recognition of factor VIII deficiency in von Willebrand's disease, as discussed on page 1179. A report of hemophilia A with autosomal dominant transmission<sup>169</sup> appears to represent a variant of von Willebrand's disease.<sup>405b</sup> In another kindred, with otherwise typical X-linked recessive inheritance, unaffected males had low factor VIII levels, an observation suggesting that they were heterozygous for genes controlling factor VIII biosynthesis.<sup>389</sup> Still other genetic variants of hemophilia A have been reported.<sup>55</sup>

### Carrier Detection

The regularity with which the abnormal gene is suppressed by the normal allele in female carriers of hemophilia varies because of the *phenomenon of random X-chromosome inactivation* (the Lyon hypothesis). Thus, although the mean concentration of factor VIII in the plasma of heterozygous carrier females is approximately 50% of that present in normal women,<sup>32,108,261,297</sup> observed values scatter widely around this mean. Levels observed in carriers often overlap with those of the normal population,<sup>297</sup> but this is partly due to the large error of assay methods, and the wide range of factor VIII levels in normal persons.<sup>297</sup> One study suggested that assay of factor VIII will detect with reasonable accuracy about 75% of the true carriers in a potential carrier population.<sup>319</sup> Others have reported figures on the order of 35%, which

are more consistent with the experience of most investigators.<sup>406</sup>

Although the demonstration of subnormal levels of factor VIII by means of the usual assay methods strongly suggests the presence of the carrier state, the converse statement cannot be made with equal certainty, i.e., the presence of normal levels of factor VIII does not reliably exclude the carrier state.<sup>319</sup> Furthermore, pregnancy and anovulatory medications may increase the levels of factor VIII in carrier females<sup>27</sup> (page 421). Immunologic assays of factor VIII greatly improve the accuracy of carrier detection,<sup>27,102,326,432</sup> but are not as yet generally available.

### Hemophilia in the Female

Hemophilia has been well documented in human females.<sup>59,135,140,270,424</sup> The most common form is that seen in a minority of *heterozygous carriers*, discussed above, in whom X-chromosome inactivation occurs at an unusually early stage of embryogenesis and results in unusually low levels of factor VIII.<sup>32</sup>

A second cause of female hemophilia is a mating between an affected male and a carrier female (Fig. 37-1, generation IV, number 10).<sup>253,400</sup> One half of the female offspring of such a match would inherit two abnormal X chromosomes, one from the father and one from the mother. At one time it was suspected that such *homozygous female hemophilia* might be lethal and inhibit the development of the embryo. That this is not true was first suggested by the successful experimental production of hemophilia in a female dog.<sup>151</sup> Homozygous hemophilia has now been well authenticated in several women,<sup>253,400</sup> and resembles the disorder seen in affected males in all respects.

In several other instances of female hemophilia the disorder appeared to have developed *spontaneously*,<sup>10,237</sup> presumably as the result of a newly mutant gene. Rarely a *chromosomal abnormality* resulting in a hemizygous genotype in the female may be responsible, eg, 46 XX/45 X mosaicism,<sup>138</sup> 46 XY karyotype.<sup>273</sup>

## Incidence

Hemophilia A has been recognized in all areas of the world where adequate information is available.<sup>126</sup> The disorder seems to be rare among Chinese, and is uncommon in Negroes.<sup>65, 289</sup> Earlier data concerning the occurrence of hemophilia A certainly included persons with hemophilia B; many male patients afflicted with von Willebrand's disease and other persons with hereditary coagulation disorders probably were included as well. No doubt these data excluded many of the less severe cases.

Hemophilia A is the commonest of the hereditary coagulation disorders. The absolute incidence of the disorder has not been defined accurately, the best available estimates range from 1 in 20,000 to as high as 1 in 10,000 persons.<sup>190, 317, 369</sup>

The relative incidence of hemophilia A, as compared to the various other hereditary co-

agulation disorders, varies, depending on the particular population studied, but several major surveys, which reflect largely northern European and American populations, have revealed that an average of 80% of the cases of hereditary coagulation disorders were hemophilia A.<sup>274</sup>

## Clinical Manifestations

The most dramatic manifestation of hemophilia A is exsanguinating hemorrhage from a trivial traumatic injury. However, the most characteristic bleeding manifestations, such as hemarthrosis, often develop without significant trauma, and their frequency and severity generally are related to the blood level of factor VIII.<sup>39, 67</sup>

Three categories of severity may be arbitrarily distinguished (Table 37-3): (1) *Severe deficiency* (factor VIII level 0 to 2% of normal), which is clinically manifested by re-

**Table 37-3. Clinical and Laboratory Findings in Hemophilia A and Hemophilia B**

Severity	Factor VIII or IX Level (% of Normal)	Clinical Picture	Coagulation Tests		
			Coagulation Time	Prothrombin Consumption Test	Partial Thromboplastin Time
Severe	0-2	Hemarthrosis and spontaneous bleeding severe and frequent, crippling common	Prolonged	Abnormal	Prolonged
Moderate	2-5	Spontaneous bleeding and hemarthroses infrequent crippling uncommon serious bleeding from trivial injuries	Normal	Variable	Prolonged
Mild	5-25	Spontaneous bleeding and hemarthroses uncommon, unsuspected and serious bleeding from traumatic injuries and surgery, diagnosis may be missed	Normal	Normal	Variable
* Subhemophilia*	25-50	Moderate bleeding following major trauma or surgery, diagnosis often missed	Normal	Normal	Usually normal



peated and severe hemarthroses that almost invariably eventuate in crippling; such severe cases often are referred to as "classic" hemophilia. (2) *Moderate deficiency* (factor VIII level 2 to 5% of normal), which is associated with less frequent and less severe hemarthroses and seldom results in serious orthopedic disability. (3) *Mild deficiency*<sup>152</sup> (factor VIII level 5 to 25% of normal), in which hemarthroses and other spontaneous bleeding manifestations may be absent altogether, although serious bleeding may follow surgical procedures or traumatic injury.<sup>49</sup> Some authors include a fourth category termed "*subhemophilia*"<sup>67</sup> (factor VIII level 25 to 50% of normal), which results in no unusual bleeding except when the patient experiences serious trauma or a major surgical procedure.

### Hemarthrosis

Hemarthrosis is the most common, the most painful, and the most physically, economically, and psychologically debilitating manifestation of the hereditary coagulation disorders.<sup>50,91,200</sup>

**PATHOPHYSIOLOGY.** Bleeding presumably originates from the synovial vessels, and develops spontaneously or as the result of imperceptible or trivial trauma. Hemorrhage occurs into the joint cavity or into the diaphysis or epiphysis of the bone. In the *acute stage*, the synovial space is distended with blood. Muscular spasm further increases the intrasynovial pressure. Hemorrhage into the periarticular structures is a common complicating feature, and occurs most frequently around small joints.

The joint may regain normal function following the first episodes of hemarthrosis. More frequently, however, the absorption of intra-articular blood is incomplete, the retained blood produces chronic inflammation of the synovial membrane, and the joint remains swollen, tender, and painful for months or years. This has been referred to as the second stage of hemarthrosis, or the *stage of panarthrititis*.

Acute hemarthroses almost invariably

recur from time to time. With each recurrence, the synovium becomes progressively more thickened and vascular; folds and villi, which predispose to synovial injury during even minimal activity, may be formed. Together with the weakening of the periarticular supporting structures, this process predisposes the joint to recurrent episodes of bleeding. Repeated bouts of hemarthrosis, with the associated subchondral and synovial ischemia, result in progressive loss of hyaline cartilage, particularly at the margins of the joint. Large punched-out areas of destruction are sometimes produced by subchondral hemorrhages and, in the cancellous structure of the bone, cavitation may be caused by intraosseous hemorrhage. Through disuse, diffuse demineralization of the involved bones also may occur. Subperiosteal hemorrhages are not common.<sup>404</sup>

The *terminal stage* of hemarthrosis is not sharply differentiated from the panarthritic stage. In the larger joints, fibrous or bony ankylosis is common, but in the smaller articulations complete destruction may take place because of the weaker joint structure and the thinner cortices of the smaller bones.<sup>121</sup> Other permanent sequelae of hemarthrosis include atrophy and proliferation of bone, roughening of the articular surfaces with lipping and osteophyte formation, bone necrosis and cyst formation, stunted growth as the result of interference with the nutrition of the bone, and accelerated development and overgrowth of the epiphyses from excessive flow of blood to the growing epiphyses.<sup>50</sup>

**CLINICAL PICTURE.** The earliest symptom of hemarthrosis is pain, which in the acute form may be excruciating. Physical examination reveals muscle spasm and limitation of motion of the affected joint, which is usually held in a position of flexion. The joint may be warm and grossly distended and discolored, but external evidence of bleeding may be minimal or absent in chronically damaged large joints because of thickening of the articular capsule. Generally, only one joint is involved at a time, although bleeding

may develop simultaneously in two or more joints. The ankle frequently is the earliest joint involved, but the knee is the one most commonly affected and the one most often permanently crippled. Other joints that may be involved are the elbow, the hips,<sup>428</sup> wrists, shoulders, small joints of the hand and feet, the vertebral articulations,<sup>393</sup> and the temporo-mandibular joint.<sup>49</sup>

**RADIOLOGIC FINDINGS.** Radiologic changes range from a slight increase in the soft tissue shadows in the joint space, which results from distention or thickening of the synovium or joint capsule, to the aforementioned destructive changes that radiologically resemble advanced osteoarthritis<sup>330</sup> (Fig. 37-2).

#### *Subcutaneous and Intramuscular Hematomas*

Large ecchymoses and subcutaneous and intramuscular hematomas are common in hemophilia A, and characteristically spread within fascial spaces and dissect deeper structures. Subcutaneous bleeding may extend over as much as half the body and does so in a characteristic manner. At the site of origin the tissue is hard, indurated, raised, and purplish black. From this center, the hemorrhage extends in all directions "like ripples on a pond,"<sup>40</sup> with each successive concentric extension less deeply colored. The point of origin of the hemorrhage may be entirely absorbed, while the margin is still progressing. Intramuscular and subcutaneous hematomas may produce leukocytosis, fever, and severe pain, in the absence of significant discoloration of the overlying skin.

Hematomas may produce serious consequences from the *compression of vital structures*. Bleeding into the tongue, throat, or neck may develop spontaneously and is especially dangerous, since it may compromise the airway with surprising rapidity.<sup>227</sup> Gangrene may result from pressure on arteries,<sup>246</sup> and ischemic contractures are common sequelae of hemorrhage into the calves or forearms, eg, Volkmann's contracture. Peripheral nerve lesions of varying severity are common complications of hemorrhage into joints or



Fig 37-2 Roentgenogram of elbow and knee joints in a patient with hemophilia A. Thickening of synovium with deposition of calcium is shown in A and A', increased intercondylar notch in B, increased density decreased interarticular space in A, B, and C, and lipping along the borders of the joint surfaces in C.

muscles,<sup>365</sup> eg, femoral nerve compression due to hematomas of the iliacus.<sup>146</sup>

**PSOAS AND RETROPERITONEAL HEMATOMAS.** Spontaneous hemorrhage into internal fascial spaces and muscles of the abdomen is common in hemophilia A. Bleeding into or around the iliopsoas muscle produces pain of progressively increasing severity and tenderness, and, when on the right side, may closely simulate acute appendicitis. Partial or complete involvement of the femoral nerve may take place with the development of pain on the anterior surface of the thigh. The "psoas sign" is positive, and the hip is held in partial flexion. Paresthesias, partial or complete anesthesia, and ultimately weakness or paralysis of the thigh extensors with eventual muscular atrophy may ensue.

Retroperitoneal hemorrhage also is common, and may be mistaken for appendiceal abscess. Intraperitoneal hemorrhage and bleeding may occur elsewhere in the abdomen and may simulate virtually any acute intra-abdominal condition.

### *Gastrointestinal and Genitourinary Bleeding*

Hemorrhage from the mouth, gums, lips, and tongue is common and often serious. The eruption and shedding of deciduous teeth usually occurs without abnormal bleeding, but may be accompanied by hemorrhage that lasts for days or weeks. Epistaxis occurs in almost all patients, and may be of exsanguinating proportions.

Hematemesis, melena, or both are not uncommon. The source of the blood usually is in the upper gastrointestinal tract, and in the majority of patients in whom bleeding is persistent or recurrent it is found to originate from some organic lesion, most commonly a peptic ulcer or gastritis. Hemorrhage may be accompanied by abdominal pain, distention, increased peristalsis, fever, and leukocytosis. In one of our patients, gastrointestinal bleeding followed the ingestion of a peach stone. Intramural bleeding into the intestinal wall may result in intussusception or obstruction.

Hematuria, although much more common than gastrointestinal bleeding, is less often the result of a demonstrable pathologic condition in the genitourinary tract. The bleeding may arise in the bladder or in one or both kidneys, and may persist for days or weeks.<sup>304a</sup> When the hemorrhage begins to lessen and clots begin to form, ureteral colic may develop.

### *Traumatic Bleeding*

Patients with coagulation disorders seldom bleed abnormally from small cuts, such as razor nicks. Following larger injuries, however, hemorrhage out of all proportion to the extent of the injury is characteristic. This may persist as a slow, continuous oozing for days, weeks, or months, or may be massive and life-threatening.

*Delayed bleeding* is common. Thus, although hemostasis following an injury or a minor surgical procedure may appear to be entirely adequate, hemorrhage, often of sudden onset and serious proportions, may develop several hours or even days later. This phenomenon apparently is the result of the

fact that the processes of primary hemostasis are only temporarily effective. Delayed bleeding may occur in mild hemophilia, and is a significant hazard following minor surgical procedures, particularly those performed on an outpatient basis, eg, tooth extractions, tonsillectomy.

*Venipuncture*, if skillfully performed, is without danger to the hemophiliac because of the elasticity of the venous walls. If venipuncture is traumatic, a pressure dressing may prevent further complications. Subcutaneous, intracutaneous, and small intramuscular injections seldom produce hematomas if firm finger pressure is maintained for at least five minutes. *Lumbar puncture* can usually be carried out without serious risk if performed expertly but many prefer to administer factor VIII beforehand.

### *Miscellaneous Clinical Manifestations*

Infants are usually asymptomatic, since they are insulated from trauma<sup>13</sup>; hematomas are first seen when the child becomes active, and hemarthroses seldom develop until he begins to walk. Occasionally evidence of this disorder is not seen until the patient reaches teenage or young adult life.<sup>93</sup> Spontaneous hemorrhage may be cyclic in nature. Petechiae, which are characteristic of disorders of platelets and blood vessels, are rare in hemophilia, but have been noted in severely affected patients at the "peak" of an "attack" of bleeding.<sup>49</sup> Hemorrhage from the umbilical cord or stump is unusual, but prolonged bleeding after circumcision brought hemophilia to the attention of the ancient Hebrews. Pulmonary and pleural bleeding are uncommon,<sup>16</sup> although mediastinal and pleural shadows have been noted in roentgenograms, and presumably originate from fresh or old hematomas.<sup>93</sup> Intraocular hemorrhage is uncommon, but bleeding into the orbit and conjunctiva occurs frequently. Spontaneous rupture of the spleen has been reported.<sup>73</sup>

*Intracranial bleeding* has been reported in 2.5 to 7.8% of the patients in various series and tends to occur in younger hemophiliacs, especially in relation to head trauma.<sup>364,365</sup>



Fig 37-3 Roentgenogram showing pseudotumor and destruction of the ilium in a patient with hemophilia B

It is frequently subdural, epidural, or intracerebral in location. Subarachnoid bleeding occurred least commonly in the reported patients but had the best prognosis. Hemorrhage also may develop in the spinal cord or spinal meninges. The overall mortality from intracranial bleeding was 70%<sup>364</sup> in one series, but more recent experience suggests a much more favorable prognosis.<sup>351a,365</sup>

*Hemophilic cysts* are a serious complication and most commonly develop in patients with severe hemophilia A or B. Also known as hemophilic "pseudotumors," such cysts represent gradually expanding blood-filled loculations that apparently originate from hemorrhages into confined subperiosteal, tendinous, or fascial spaces (Fig. 37-3).<sup>41</sup> The osmotic pressure created by breakdown products of blood in such confined spaces may produce further influx of fluid, this, together with recurrent bleeding, explains the cyst's slowly progressive increase in size and its ability to erode contiguous structures. Such cysts most commonly develop in the thigh, and many destroy bone as well as the soft tissues as their size increases. These lesions can be more readily prevented than treated. They usually require radical surgical procedures, eg, extensive resections or amputation. Even with optimal supportive therapy, these procedures

often are unsuccessful and are frequently complicated by infection.

*Wound healing* is often slow in hemophiliacs. This is probably due to continued or intermittent hemorrhage or complicating infection. There is no evidence that factor VIII has any specific role in wound healing, as has been postulated for fibrinogen and factor XIII (page 440).

The *psychologic effects* of hemophilia can be many and severe, and often influence the course of the illness.<sup>7</sup> For example, there may be increased risk-taking as a neurotic response to the disease. At times of emotional stress, spontaneous bleeding has been observed. Parental guilt feelings of both a conscious and unconscious nature are common, and the emotional interaction between carrier mothers and affected males often is abnormal.

### Course and Prognosis

It has often been observed that hemophiliacs improve at or after adolescence.<sup>37,77,313</sup> An analysis of 113 cases in 1937 indicated, however, that the more benign course of older hemophiliacs can be attributed to: (1) death in early life of those having the most severe cases; (2) the passing of the teething period; (3) the onset of the "years of discretion"; and (4) increasing inactivity as the result of permanent joint deformities. In the same study it was calculated that the life expectancy of hemophilic babies was one-twelfth of that of the normal. The greatest number of deaths (23%) were due to exsanguination following surgical procedures.

In recent years, prognosis in severe hemophilia has improved greatly. With proper treatment a nearly normal life span can be expected, and the crippling sequelae of the disease can be minimized.

### Laboratory Diagnosis

Basic hematologic examinations reveal nothing characteristic in hemophilia A. The presence or absence of anemia depends on the severity and frequency of bleeding. Blood regeneration is usually rapid when hemor-

rhage has ceased. Neutrophilia may accompany the bleeding. As in other instances of posthemorrhagic anemia (Chapter 13), the bone marrow reflects the response to blood loss. The megakaryocytes are normal or increased in number.<sup>62</sup>

### Screening Tests

The *partial thromboplastin time* usually is prolonged in hemophilia A (Table 37-4). The results of this test vary, depending on the factor VIII level in the individual patient (Table 37-3), but abnormal results are usually obtained if the factor VIII levels are less than 20 to 25% of normal. The abnormality in the PTT can be normalized by the admixture of the patient's plasma with approximately 20% of normal plasma or another reagent that contains factor VIII (Table 37-4). This "corrective" effect provides the basis for many simple "presumptive" tests for hemophilia A.<sup>61,219</sup> Such qualitative techniques are not as reliable as the TGT, however.

The *bleeding time* may be prolonged and the reaction to the tourniquet test may be positive in an occasional patient with hemophilia.<sup>61</sup> In some patients, these findings appear to be correlated with acute exacerbations of bleeding; in others, they may be attributable to the use of aspirin or to associated platelet dysfunction<sup>167b</sup> (page 1129).

The *platelet count* usually is normal or elevated, and clot retraction is normal. Thrombocytosis may reflect a response to acute or chronic hemorrhage, but in many hemophiliacs it has been found in the absence of significant bleeding. It was suggested that this may represent hemostatic "compensation,"<sup>17</sup> but there is little supporting evidence for this view. Morphologic platelet abnormalities have been reported, but are probably not significant.<sup>126</sup>

### Ancillary Tests

The *coagulation time* of whole blood and the results of tests of *prothrombin consumption* vary, depending on the severity of the factor VIII deficiency (Table 37-3). The

clotting time may be 24 hours or longer, and may show spontaneous irregular variations. It will be normal, however, if the factor VIII level exceeds 1% of normal.<sup>361</sup> One-stage tests of prothrombin consumption are a little more sensitive than the clotting time, but will give normal values if the factor VIII level is above 2 to 4% of normal. With the two-stage technique, normal results are obtained when the factor VIII level is above 1% of normal.<sup>361</sup>

The results of the *thromboplastin generation test (TGT)* may be abnormal, due to deficient function of the adsorbed plasma reagent (page 1057). The finding of such a "plasma defect" together with a normal prothrombin time, which excludes deficiency of factor V, is virtually diagnostic of hemophilia A in a patient with a hereditary disorder (Fig. 33-6, page 1059). The *plasma recalcification time* is greatly prolonged. Like the PTT, the TGT and the recalcification time may be corrected by the addition of various "reagents" containing factor VIII (Table 37-4). The thrombin time and Stypven time are normal.

### Factor VIII Assay

Factor VIII assay is a relatively simple technique, which, because of its importance, is becoming available in an increasingly large number of hospitals. Two-stage,<sup>41 210,265,301</sup> one-stage,<sup>163 426</sup> and micro<sup>108</sup> methods are suitable for diagnosis (page 1061). The one-stage techniques are the most widely used since they are somewhat simpler to perform, but they require a supply of plasma from a known hemophiliac, which may be a disadvantage in small laboratories. Such plasma is available commercially. Artificial substrates have been advocated as substitutes for native factor VIII deficient plasma.<sup>82,120</sup>

There is no stable, generally available reference standard for the assay of factor VIII.<sup>14,210</sup> The most satisfactory standard appears to be a lyophilized preparation of concentrated factor VIII,<sup>14</sup> but lyophilized citrated plasma, which is available commercially, or a pool of frozen citrated plasma

### Table 37.4. Laboratory Findings in the Hereditary Coagulation Disorders

Disorder	Bleeding Time (1)	PTT	Prethrombin Time	Coagulant on Trog (2)	Pathologic Consumption (2)	Thrombin Time	Stypsin Time	TG	In Vivo Consumption Abnormalities	Ancillary Tests
Hemophilia A	N	A	N	A	A	N	N	PD	NP aNP NS aNS aP	
Hemophilia B	N	A	N (18)	A	A	N	N	SD	NP NS aP aNP aNS	
von Willebrand's Disease (3)	A	UA	N	UA	UA	N	N	aPD	NP aNP NS aNS aP	Platelet count on tests, new factor VIII synthesis & desmopressin-induced platelet aggregation
XI deficiency	UA	A	A	A	N	A	A	N	NP aNP aP NS aNS	Platelet function tests
XII deficiency (4)	N	VA	A	UN	N	A (4) (5)	VA	N	NP aNP aP (4) NS aNS	5-Br-potometer assay, coagulant effects of various venoms
Factor V deficiency	UN	A	A	VA	VA	N	A	N	NP aP NS aNP aNS	2-Stage prothrombin assay
Factor VII deficiency	N	N	A	N	N (7)	N	N	N	NP NS aP aNP aNS	
Factor X deficiency	N	A	A	A	A (7)	N	A (8)	SD	NP NS aP aNP aNS	
Factor XI deficiency	N	A	N	A	A	N	N	mild PD and SD	NP aNP NS (8) aNS aP	Clotting & contact activation tests
Factor XIII deficiency	N	A	N	A	A	N	N	mild PD and SD	NP aNP NS (8) aNS aP	Clotting & contact activation tests
Factor XIII deficiency	N	N	N	N	N	N	N	N	NP aNP aP NS aNS (8)	Clot solubility tests

N = normal    A = abnormal    O = deficient    P = plasma    S = serum    V = variable    W = usually    X = adsorbed with  $\text{Al}(\text{OH})_3$  or similar substance    O = aged in oxalate or EDTA

The platelet count and clot retraction are normal in all uncomplicated cases. The platelet count and clot retraction are normal in all uncomplicated cases.

[illegible]

(2) Tests of prothrombin consumption and coagulation time give abnormal results only in severe deficiencies.

(Table 37.3).

(3) Congestive abnormal lives are due to deficiency of factor VIII

(4) Patient's plasma may inhibit normal coagulation

(5) Abnormality may be corrected by increasing calcium concentration and may be magnified by diluting the precipitant solution.

(b) Findings are significantly different in some variants

1273 Results of one-stage techniques are unacceptable

(10) Correction variable in degree

and from the Fisheries Board—£1.5

carefully collected from normal subjects serves as well under most circumstances. It must be recognized that the factor VIII assay has a relatively large error even in expert hands, and when borderline values are obtained the assay should always be repeated.

### Differential Diagnosis

The diagnosis of hemophilia A is seldom difficult. This is especially true in the severely affected patient in whom repeated and often serious hemorrhagic manifestations, including characteristic ones such as hemarthrosis, will be clearly apparent early in life. It is noteworthy that hemarthrosis with significant orthopedic disability is rare in coagulation disorders other than hemophilia A and hemophilia B.

In patients with the *milder forms of the disorder*, however, failure to recognize the existence of a disease or to make the correct diagnosis is more likely. In such patients, there may be little spontaneous bleeding, and the family history tends to be vague or negative. As pointed out earlier (Table 37-3) the coagulation time may be normal in such patients. Because the diagnosis of hemophilia is often erroneously equated with a prolonged coagulation time, a normal value may be misleading. It must be emphasized that mildly affected hemophiliacs still are prone to hazardous hemorrhage following trauma or during surgical procedures.<sup>291</sup> Neither the coagulation time nor tests of prothrombin consumption can be relied upon to exclude the possibility of hemophilia. In the mildly affected patient, even the PTT and the TGT may be normal, and specific assays must be carried out to confirm or to exclude the diagnosis of hemophilia.<sup>359</sup>

The results of screening tests (Table 37-4) usually are sufficient to exclude the possibility of *acquired hemorrhagic disorders* associated with serious bleeding. Such disorders are seldom associated with a prolonged PTT and a normal prothrombin time, a combination that strongly suggests a hereditary disorder or an inhibitor (page 1208). Among the *hereditary disorders* characterized by this

combination of findings (hemophilia A, hemophilia B, deficiencies of factors XI and XII), factor XII deficiency can be readily excluded, since it is not associated with bleeding (page 1179). Factor XI deficiency in males may mimic mild hemophilia, and hemophilia B is clinically identical to hemophilia A. Both factor XI deficiency and hemophilia B must be distinguished from hemophilia A in the laboratory; the most useful, generally available test for this purpose is the TGT (Fig. 33-6, page 1059). A definitive diagnosis is of great importance since various concentrates, rather than plasma, are now used in the treatment of these disorders.

Von Willebrand's disease in males may be indistinguishable from mild hemophilia A associated with a prolonged bleeding time, even if the latter abnormality is an inconsistent finding. Confirmatory tests for von Willebrand's disease, as discussed on page 1182, are required to make this distinction.

The bleeding manifestations in hemophilia may simulate a great variety of conditions. However, this has resulted in serious confusion only when the correct diagnosis has not been considered, and appropriate laboratory studies have not been ordered. Thus, a deep hematoma may be mistaken for a suppurative condition and surgical drainage may be attempted. Bleeding into a small joint may produce a clinical and roentgenologic picture suggesting sarcoma<sup>124</sup>; when larger joints are involved, tuberculosis, arthritis, Perthe's disease, or syphilis is simulated. Bleeding elsewhere may suggest local causes, eg, kidney tumor, pulmonary disease, peptic ulcer.

*Intra-abdominal bleeding* raises particularly serious diagnostic and therapeutic problems in hemophilia, even when the hemophilia has been accurately diagnosed. Thus, hemorrhage into the psoas, when on the right side, may simulate acute appendicitis so closely that, in the opinion of many experienced workers, there is no reliable way to differentiate between the two.<sup>41</sup> A retroperitoneal hematoma may be mistaken for an appendiceal abscess. Intraperitoneal hemorrhage and bleeding into and around other viscera may simulate perforating peptic ulcer, bowel obstruction, or

virtually any other acute intra-abdominal condition.

## Hemophilia B

That hemophilia represents at least two different disorders was recognized in 1947.<sup>292</sup> Hemophilia B (Christmas disease, factor IX deficiency, plasma thromboplastin component [PTC] deficiency) was first clearly distinguished from hemophilia A by Aggeler et al in 1952.<sup>6,43,319</sup> Hemophilia A is from four to eight times more common than hemophilia B.<sup>39,274</sup>

### Pathophysiology

Hemophilia B does not represent a true deficiency of factor IX in the usual case. As in hemophilia A, the majority of patients with this disorder have normal plasma levels of factor IX-related antigens that neutralize heterologous antibodies obtained from rabbits.<sup>255</sup> The absence of cross-reacting material has been documented, however, in a few patients with hemophilia B.<sup>340</sup> In this form of the disorder, which has been termed *hemophilia B Leyden*,<sup>407</sup> the clinical manifestations tend to diminish with advancing age in association with a rise in the factor IX level from as low as 1% in childhood to levels of 20% or more in adult life. This remarkable variant of hemophilia B resembles the disorder encountered in the Tenna kindreds.<sup>260</sup>

In another variant of hemophilia B, the prothrombin time is prolonged when performed with bovine brain thromboplastin.<sup>50,179,255</sup> This disorder has been termed *hemophilia Bm*<sup>399</sup> and is characterized by the presence in the plasma of CRM that neutralizes both homologous and heterologous antibodies to factor IX.<sup>75,253</sup> The abnormality of the ox brain prothrombin time is proportional in degree to the plasma level of CRM in both affected males<sup>179,399</sup> and carrier females,<sup>50</sup> and presumably is the result of inhibition of the extrinsic pathway of coagulation by the abnormal analog of factor IX. However, there is little evidence that factor

IX normally is involved in the extrinsic pathway of coagulation (page 425), and the mechanism of this inhibitory effect is obscure.<sup>103</sup> An apparently unrelated inhibitor appeared to be the cause of lifelong factor IX deficiency in one patient.<sup>385</sup>

### Genetic Features

Hemophilia B is inherited as an X-linked recessive trait, but the locus on the X chromosome of the gene controlling factor IX production is remote from that involved with factor VIII biosynthesis.<sup>17,96</sup> Certain differences between the genetic features of the two disorders have been documented. Thus, patients with hemophilia B who do not have a clearcut family history ("spontaneous" cases) are relatively less common,<sup>100,374</sup> and abnormal hemorrhage in heterozygous female carriers is more common than in hemophilia A. In one series of 45 obligatory carriers,<sup>258</sup> the mean factor IX level in the plasma was 33%; 40 of the group had levels below 60%, and in 10 the levels were below 25%. As a consequence, carrier detection is somewhat easier in this disorder than in hemophilia A.<sup>107,406</sup> Factor IX levels below 10% have been documented in only six women, including two with chromosomal abnormalities.<sup>50,267a</sup>

### Clinical Features

Severely affected patients (those with factor IX levels below 2%) are less common in hemophilia B than in hemophilia A,<sup>40</sup> but the clinical manifestations of the two disorders are identical.

### Laboratory Diagnosis

The laboratory diagnosis of hemophilia B involves the same approach (Fig. 33-6, page 1059) and methods as those described above for the recognition of hemophilia A (Table 37-3). The screening tests reveal similar abnormalities in the two disorders, except that the prothrombin time is abnormal in the Bm



variant, when either bovine brain or Thrombotest is employed as the thromboplastin.<sup>399</sup> In an occasional subject, the bleeding time is prolonged.<sup>51</sup>

Hemophilia A may be distinguished from hemophilia B by the thromboplastin generation test; in the latter disorder a "serum" defect is apparent.<sup>262</sup> One-stage<sup>107,109,380</sup> and two-stage<sup>46,57</sup> assays for factor IX employ the same principles as those discussed for factor VIII.<sup>37,109</sup> Methods for preparing an artificial substrate plasma that is deficient in factor IX also have been described.<sup>380</sup>

## Factor XI Deficiency

Factor XI deficiency (plasma thromboplastin antecedent [PTA] deficiency) was first recognized by Rosenthal et al in 1953.<sup>343</sup> This disorder was at first thought to be transmitted as an autosomal dominant trait, with a high degree of penetrance but with variable expression. Later studies suggested that it is transmitted as an incompletely recessive autosomal trait manifested either as a major defect in homozygous individuals with factor XI levels below 20% or as a minor defect in heterozygous individuals with levels ranging from 30 to 65%.<sup>229,321</sup> The incidence of this disorder, compared to that of other hereditary coagulation disorders, varies widely, ranging from 1 to 18% and even more<sup>89</sup> of surveyed cases. This variation may be attributable to a particularly high frequency of the disorder in persons of Jewish extraction.<sup>321</sup>

### Pathophysiology

The pathophysiology of factor XI deficiency is poorly understood. In one study of 10 cases, material that cross-reacted with a heterologous antibody to semipurified factor XI was lacking.<sup>128</sup>

### Clinical Features

The clinical manifestations of factor XI deficiency are milder than those of either

hemophilia A or B. As a rule, spontaneous bleeding is rare, and hemorrhage usually occurs only following trauma or after a surgical procedure. Hemarthrosis is quite uncommon, but delayed bleeding has been a particularly treacherous feature in some of these patients.<sup>343</sup> Hemorrhagic manifestations were absent in two patients.<sup>115,395</sup>

### Laboratory Diagnosis

In the homozygous form of factor XI deficiency,<sup>105</sup> the PTT and the coagulation time of whole blood are prolonged, and prothrombin consumption is deficient. These abnormalities can be corrected by giving small amounts of normal serum or plasma from which the vitamin K-dependent coagulation factors have been absorbed (Table 37-4). The clotting time and PTT may be nearly normal when performed in silicone-coated glassware. The bleeding time rarely is prolonged.<sup>426</sup>

In most patients, factor XI levels in the plasma are in the range of 3 to 15% of normal. More severely affected patients are rare. In persons with the mild form of the disorder, the coagulation time and prothrombin consumption usually are normal; even the PTT often is normal.<sup>89</sup> Abnormalities in the plasma of such mildly affected patients may be abolished by freezing.

Deficient prothrombinase formation in the thromboplastin generation test results from abnormalities of both the serum and plasma reagents. The most clearcut abnormalities are obtained when both reagents are derived from the blood of the patient. Approximately 33% of factor XI is adsorbed from plasma by aluminum hydroxide.<sup>278</sup>

Several quantitative assays for factor XI have been described.<sup>278,320</sup> Artificially deficient substrate plasma<sup>178</sup> has proved useful, but such substrates also are deficient in factor XII and possibly the Fletcher factor as well. The celite eluate test is a relatively cumbersome method for the diagnosis of factor XI deficiency.<sup>281</sup> It would appear that, in the assay of factor XI, totally satisfactory results depend on the availability of fresh plasma from a severely affected patient.<sup>113</sup>

## Disorders of Fibrinogen

### Hereditary Afibrinogenemia

Since hereditary afibrinogenemia was first described by Rabe and Salomon in 1920,<sup>315</sup> more than 60 cases have been reported.<sup>197,423</sup> The disorder is inherited as an autosomal recessive trait. Although siblings have been affected, the disorder has not been documented in consecutive generations of the same family. In some instances, moderate hypofibrinogenemia has been demonstrated in presumed heterozygotes,<sup>225,430</sup> but, in most kindreds, carriers cannot be identified by laboratory means. Parental consanguinity has been present in over 50% of reported kindreds.

### Pathophysiology

Hereditary afibrinogenemia appears to be the result of deficient biosynthesis of fibrinogen. Fibrinogen cannot be identified in the blood of affected patients by means of electrophoretic methods or by precipitation with heat or chemicals, although immunochemical methods have sometimes revealed trace amounts ( $<5$  mg/dl).<sup>143</sup> Platelet-associated fibrinogen also is deficient, an observation that presumably explains the abnormalities of platelet function seen in afibrinogenemia (page 1127). Fibrinolytic activity<sup>12</sup> and the turnover rate of infused fibrinogen<sup>143</sup> are normal in this disorder.

### Clinical Features

The hemorrhagic tendency is present from birth and bleeding from the umbilical cord and following circumcision may be profuse. Other common manifestations include bleeding following slight trauma, subcutaneous hemorrhages, epistaxis, and excessive bleeding during the eruption and loss of deciduous teeth. Wound healing may be defective. Despite the complete incoagulability of the blood, patients with this disorder have enjoyed long periods of freedom from hemorrhage and are likely to have much less disability than those with hemophilia A.<sup>315</sup>

Hemarthroses are uncommon, and, in affected women, menses often are normal.

### Laboratory Diagnosis

In the usual case, the blood is incoagulable. The clotting time, PTT, prothrombin time, and thrombin time are grossly abnormal. The TGT and tests of prothrombin consumption give normal findings. The bleeding time is prolonged in approximately 50% of these patients, and more definitive tests provide evidence of platelet dysfunction in the majority of those affected (page 1127). These abnormalities can be corrected *in vivo* and *in vitro* by small amounts of fibrinogen. Because of the total absence of fibrinogen, the erythrocyte sedimentation rate (page 125) usually is nil in this disorder.

### Hereditary or Constitutional Hypofibrinogenemia

In this poorly defined disorder, plasma fibrinogen levels range from 20 to 100 mg/dl.<sup>313,326,331</sup> Hemorrhage is infrequent and rarely is severe. In contrast to afibrinogenemia, symptoms are seldom seen in infancy, and all affected patients apparently have survived into adult life.<sup>326</sup> Both autosomal dominant<sup>296</sup> and autosomal recessive<sup>221,313</sup> inheritance have been described,<sup>331</sup> and several consecutive generations of a family have been affected. The laboratory findings in hereditary hypofibrinogenemia resemble those already described for hereditary afibrinogenemia (Table 37-4) except that the abnormalities are less marked. Some reports of hypofibrinogenemia in the earlier literature may have represented dysfibrinogenemia<sup>163,195</sup> or possibly heterozygous carriers of afibrinogenemia. There is little information concerning the pathophysiology of the disorder, and its status as a clearcut entity has been questioned.<sup>326</sup>

### The Hereditary Dysfibrinogenemias

The inheritance of a qualitatively abnormal fibrinogen was first clearly documented by

Menaché in 1963 (fibrinogen *Paris I*<sup>252</sup>). Since that time, more than 20 different abnormal fibrinogens have been described,<sup>197,326,328</sup> all designated by the city where they were first recognized. The biochemical studies required to demonstrate that these represent different disorders are lacking in many cases, and it is possible that some reports merely represent new kindreds affected with previously described defects; eg, fibrinogen *Vancouver*<sup>163</sup> may be the same as fibrinogen *Baltimore*.<sup>22</sup>

With one exception,<sup>118</sup> the hereditary dysfibrinogenemias are inherited as incompletely dominant autosomal traits. Where information is available, the plasma of heterozygotes has been found to contain both normal and abnormal fibrinogens.<sup>326,414</sup>

### Pathophysiology

The dysfibrinogenemias are the result of qualitative abnormalities of the fibrinogen molecule. Although only preliminary information is available concerning the pathophysiology of these disorders, thus far three different functional abnormalities have been demonstrated. These involve all three steps in the thrombin-fibrinogen reaction, ie, the enzymatic, polymerization, and stabilization steps (Fig. 10-4, page 424). They are: (1) delayed or disordered release of fibrinopeptides following the enzymatic action of thrombin (eg, fibrinogen *Baltimore*,<sup>22</sup> fibrinogen *Bethesda I*,<sup>155</sup> fibrinogen *Gießen*); (2) delayed or disordered polymerization of fibrin monomers (fibrinogen *Zürich I*<sup>113</sup> and *II*<sup>132</sup>; fibrinogen *Detroit*<sup>242</sup>); and (3) deficient cross-linking of fibrin monomers despite the presence of normal levels of factor XIII (fibrinogen *Oklahoma*<sup>162</sup>). In one kindred, a combination of disordered peptide release and retarded polymerization was demonstrated (fibrinogen *Cleveland II*<sup>328</sup>). In another,<sup>118</sup> the disorder was inherited as an X-linked recessive trait, and abnormally rapid fibrin polymerization was clinically manifested as a thromboembolic diathesis. In fibrinogen *Amsterdam*,<sup>198</sup> the abnormality in fibrin polymerization was not appar-

ent in the absence of an undefined plasma protein.

Differences in the several physicochemical and functional properties of the various abnormal fibrinogens have been demonstrated, eg, electrophoretic and chromatographic behavior, carbohydrate content, fibrin ultrastructure, corrective effects of calcium and protamine on the abnormalities in fibrin polymerization.<sup>197,326,328,412</sup> In fibrinogen *Detroit*, the biochemical defect has been elucidated at a molecular level. An extensive and brilliant investigation of this disorder by Blomback and associates<sup>56</sup> and by Mammen and coworkers<sup>242</sup> has shown the abnormality of the fibrinogen molecule to be the transposition of a single amino acid (serine for arginine on residue  $\approx 19$ ) near the thrombin-binding site on the alpha chain (Fig. 10-1, page 413).

### Clinical Features

The clinical picture of the hereditary dysfibrinogenemias is quite similar regardless of the particular fibrinogen that may be present. The majority of patients are asymptomatic.<sup>196a</sup> A mild hemorrhagic diathesis has been described in some forms,<sup>22,165,242,252</sup> and preliminary observations would suggest that wound dehiscence may be more common in some varieties of dysfibrinogenemia than in other hereditary coagulation disorders.<sup>129,253</sup> The occurrence of thromboembolic complications has been reported in several kindreds.<sup>22,195,348</sup>

### Laboratory Diagnosis

Laboratory studies (Table 37-4) reveal a variable prolongation of the prothrombin and the thrombin times. In some instances, the abnormality in the latter test may be partially corrected by the addition of  $\text{Ca}^{++}$  or protamine to the patients' plasma. Most of the abnormal fibrinogens characterized by slow polymerization act as inhibitors of the normal thrombin-fibrinogen reaction,<sup>326</sup> and in these varieties the abnormal thrombin time may be magnified by diluting the thrombin. The

PTT and the coagulation time usually are normal, but may be variably prolonged. Fibrinogen levels are normal when determined by heat or chemical precipitation techniques, or by immunologic methods, but, because of the slow coagulation of the abnormal fibrinogen, assays of coagulable protein may suggest moderate hypofibrinogenemia. Abnormal thrombelastographic tracings have been reported in patients with fibrinogens *Paris I*, *Cleveland*,<sup>34</sup> *Paris II*,<sup>34</sup> *Detroit*, and *Baltimore*.

*Fetal fibrinogen* is a qualitatively abnormal fibrinogen, and is discussed on page 420.

### Factor XIII Deficiency

Factor XIII deficiency was first recognized by Duckert in 1960.<sup>113</sup> More than 24 cases of this hemorrhagic diathesis have now been reported.<sup>9 123 234,411</sup> In most families, the defect is transmitted as an autosomal recessive trait, and consanguinity has been frequent in the affected families.<sup>161</sup> Transmission on the X-chromosome has been postulated in some kindreds.<sup>330</sup>

### Pathophysiology

Antigenic material related to factor XIII has been demonstrated in the plasma of affected persons,<sup>114,372</sup> with few exceptions.<sup>114,214</sup> However, the concentrations of cross-reacting material were subnormal. These data, which were obtained by methods that measure clot solubility as an index of functional factor XIII, suggest the presence of both a qualitative abnormality and a deficiency of the proenzyme. However, the neutralization of heterologous antibodies to factor XIII by the plasma of affected patients could not be demonstrated when factor XIII was assayed by its ability to catalyze the incorporation of monodansyl cadaverine into casein<sup>114</sup>; in fact, the neutralizing potency of the antiserum appeared to increase following interaction with factor XIII. The reason why this disorder is either "CRM-positive" or "CRM-negative," depending on the method

by which residual factor XIII is determined, is unknown.

### Clinical Features

In factor XIII deficiency, bleeding is often first noted when the umbilical cord separates, and deaths from cord hemorrhage have occurred in a number of instances.<sup>416</sup> Spontaneous hemorrhage is seldom severe, although hemarthrosis has been described. Ecchymoses and hematomas are common, but mucosal bleeding is infrequent. Post-traumatic or postsurgical hemorrhage may be serious, and without treatment may persist for days or weeks. Delayed bleeding is not uncommon. Hemorrhage into the central nervous system has been a serious or lethal complication in several patients with factor XIII deficiency, and is significantly more common in this disorder than in other hereditary coagulation disorders.<sup>416</sup> A high incidence of spontaneous abortion has been reported in affected women.<sup>123,191</sup>

Bleeding from the umbilical cord or stump, post-circumcision bleeding, wound dehiscence, and abnormal scar formation appear to be more common in individuals with factor XIII deficiency or afibrinogenemia, than in those with other hereditary coagulation disorders such as hemophilia A and B.<sup>137</sup> The reason for this is unclear, but it has been suggested, with some experimental support,<sup>21</sup> that factor XIII deficiency and afibrinogenemia result in abnormal or deficient fibroblastic proliferation.

### Laboratory Diagnosis

All the usual tests of coagulation give normal results in persons with factor XIII deficiency. The disorder can be readily demonstrated by clot solubility tests, normal clots being insoluble in 5 M urea or 1% monochloroacetic acid. Various chemical and isotopic techniques<sup>114,231,232,233</sup> are preferable to clot solubility measurements for purposes of quantitative assay of factor XIII.<sup>114</sup> The thrombelastogram characteristically shows

reduced amplitude and a faster decrease than is normally found.<sup>113</sup>

## Hereditary Prothrombin Deficiency

Hereditary hypoprothrombinemia is exceedingly rare; at most, 16 kindreds have been reported.<sup>59-97, 335</sup> The disorder is inherited as an autosomal dominant trait, and is clinically manifested as a relatively mild hemorrhagic diathesis. As with deficiencies of the other vitamin K-dependent coagulation factors, both CRM-positive and CRM-negative variants of hypoprothrombinemia have been described. The CRM-negative form is characterized by a true deficiency of prothrombin and appears to be the more common variety.<sup>140, 203, 209</sup> In the CRM-positive variant,<sup>203, 336</sup> the presence of normal amounts of antigenically competent prothrombin has been demonstrated by quantitative immunoelectrophoresis. In this disorder, termed "*constitutional dysprothrombinemia*,"<sup>338</sup> prothrombin appears to be normally consumed, but does not yield normal amounts of thrombin. Both normal and abnormal prothrombin molecules were present in the plasma of affected persons.<sup>336</sup> In one kindred,<sup>203</sup> prothrombin was activated normally by staphylocoagulase.

The laboratory findings in this disorder are summarized in Table 37-4. The prothrombin level in most patients was approximately 10% of normal. The two-stage method provides the only reliable test for true hypoprothrombinemia, since the one-stage prothrombin time may be nearly normal in some of these patients. The TGT is abnormal in some subjects, a finding that was attributed to deficient activation of factor V by thrombin (page 429).<sup>59</sup> The clotting time of whole blood may be normal or prolonged.

## Factor V Deficiency

Hereditary deficiency of factor V ("*parahemophilia*," *labile factor*, or *proaccelerin deficiency*) is an uncommon disease that was first

described by Owren in 1944.<sup>288</sup> It has been reported from various parts of the world,<sup>131, 218, 222</sup> and is transmitted as an autosomal recessive trait that produces clinical manifestations only in individuals who inherit the defective gene from both parents.<sup>215</sup> Other modes of inheritance have been implicated in an occasional kindred.<sup>351</sup> In the heterozygotes, the levels of factor V in the plasma are approximately half of normal, and the carriers are relatively easy to identify by laboratory studies. That this is generally true of coagulation disorders inherited as autosomal recessive traits is unexplained.<sup>406</sup>

### Pathophysiology

Preliminary data would suggest that the basic pathophysiologic mechanism in this disorder is deficient biosynthesis of factor V. The absence of cross-reacting material in the plasma of patients with this disorder has been demonstrated by antibody neutralization studies employing both naturally occurring homologous antibodies<sup>123</sup> and heterologous rabbit antibodies.<sup>101</sup>

### Clinical Features

The clinical manifestations of the disorder usually are relatively mild, but vary greatly even within the same family. In mildly affected patients, spontaneous epistaxis, easy bruisability, menorrhagia, and excessive bleeding after dental extractions or surgical procedures have been observed. In those who are severely affected, hematomas, spontaneous gingival bleeding, and bleeding into the gastrointestinal tract or central nervous system may occur. The disorder seldom produces bleeding in affected neonates, and has been associated with a high incidence of other congenital abnormalities.<sup>351</sup>

### Laboratory Diagnosis

The usual laboratory features of factor V deficiency are summarized in Table 37-4. The bleeding time has been prolonged in approxi-

mately one third of patients,<sup>287</sup> an observation that remains unexplained. Factor V deficiency is the only disorder in which a prolonged prothrombin time is associated with deficient function of the plasma reagent in the thromboplastin generation test (Fig. 33-6, page 1059). Because of the absorption of significant amounts of factor V by platelets, this abnormality in the TGT may be minimized or even abolished if normal platelets are used as the source of PF-3. The most clearcut abnormalities are obtained when a platelet substitute is used, and when the serum reagent is aged at 37°C for four hours.<sup>18</sup> Specific assays for factor V are based on the prothrombin time and are easy to perform. Good results may be obtained by using aged oxalated or EDTA plasma as a factor V-deficient substrate.<sup>163</sup> Several other suitable artificial substrates have been described.<sup>315</sup>

## Factor VII Deficiency

Factor VII deficiency was first described by Alexander et al<sup>8</sup> under the name of *serum prothrombin conversion accelerator (SPCA) deficiency*. Approximately 70 cases of true factor VII deficiency have now been reported.<sup>159,244,287</sup> Also known as *stable factor* or *proconvertin deficiency*, the condition is inherited as an autosomal recessive trait that produces severe deficiency in the homozygote and moderate deficiency, usually without clinical manifestations, in the heterozygote.

Because of the rarity of this disorder, data concerning its pathophysiology are scanty. In two patients, antigens presumably related to factor VII were demonstrated in the plasma.<sup>147,306</sup> However, in most other patients with this disorder, such antigenically competent factor VII appeared to be lacking.<sup>101,244</sup> These preliminary studies suggest the existence of both qualitative and quantitative forms of factor VII deficiency.

Hemorrhage is relatively more severe in patients with factor VII deficiency than in those with deficiency of factor V. Common manifestations include spontaneous epistaxes,

deep subcutaneous hematomas, genitourinary and gastrointestinal bleeding, and hemarthroses. The severity of bleeding following trauma or surgical procedures has varied to a surprising degree in this disorder, and in some instances appeared to bear little relationship to the extent of the laboratory abnormalities.

In factor VII deficiency, normal results are obtained with tests that bypass the extrinsic pathway of coagulation and factor VII (Table 37-4) (Fig. 33-4, page 424), i.e., the prothrombin consumption test, coagulation time, PTT, Stypven time, and thromboplastin generation test.

## Factor X Deficiency

Factor X deficiency was discovered independently by Telfer et al<sup>392</sup> and Hougic et al<sup>180</sup> and is also known by the surnames of the patients who were first found to manifest the defect (*Stuart*<sup>180</sup> and *Prower*<sup>392</sup>). Approximately 20 affected kindreds have since been reported.<sup>228,394</sup> Factor X deficiency is inherited as an autosomal recessive trait, and the genetic features and clinical manifestations of the disorder resemble those of factor VII deficiency, described above.

Several studies employing the techniques of immunodiffusion and antibody neutralization have established the existence of both "CRM-positive" and "CRM-negative" variants of factor X deficiency.<sup>104,307</sup> Significant differences in the laboratory abnormalities encountered in various kindreds further suggest that there are at least two different CRM-positive variants,<sup>104</sup> namely, that present in the Prower kindred<sup>392</sup> in which the Stypven time is abnormal, and factor X *Friuli*<sup>112</sup> in which it is normal. In the CRM-negative variant (the disorder present in the Stuart kindred) the Stypven time also is abnormal.<sup>180</sup> Additional differences in the results of in vitro coagulation tests, the levels of CRM, and immunoelectrophoretic patterns obtained with the plasma of various patients suggest at least two additional variants.<sup>104</sup> Because of the marked polymorphism

of this rare trait, the term *factor X defect* has been proposed.<sup>101</sup>

The laboratory findings in factor X deficiency differ from those in factor VII deficiency in that the thromboplastin generation test reveals a "serum defect." Prolongation of the Stypven time is characteristic in the Stuart and Prower variants (Table 37-4). Prolonged bleeding time has been reported in association with factor X deficiency.<sup>61</sup>

## Factor XII Deficiency

*Factor XII deficiency was discovered by* Ratnoff and Colopy during a routine preoperative measurement of the coagulation time of Mr. John Hageman, an adult who had no evidence or history of abnormal bleeding.<sup>329</sup> The disorder, subsequently named *Hageman factor deficiency*, is relatively rare, and is inherited as an autosomal recessive trait.<sup>19,106,321</sup> In one kindred the inheritance appeared to be autosomal dominant.<sup>28</sup> Chromosomal abnormalities were associated with factor XII deficiency in one patient.<sup>98</sup>

### Pathophysiology

The plasma of patients with factor XII deficiency does not contain material that reacts with antibodies to this factor, as judged by antibody neutralization, hemagglutination inhibition, and immunodiffusion.<sup>370</sup> This preliminary evidence would suggest that this disorder is due to deficient biosynthesis of factor XII.

### Clinical Features

Factor XII deficiency is usually not associated with hemorrhagic manifestations,<sup>329</sup> although minor bleeding has been reported in several of these patients.<sup>45,106,158</sup> It is noteworthy that myocardial infarction and thrombophlebitis have been observed in patients with severe factor XII deficiency,<sup>144,174</sup> and that Mr. Hageman died of thromboembolic complications.<sup>333</sup>

### Laboratory Diagnosis

The coagulant effects of contact activation are diminished or absent in plasma from subjects with factor XII deficiency.<sup>278</sup> The usual laboratory findings (Table 37-4) include prolonged coagulation time, markedly decreased prothrombin consumption, and deficient activity of both the serum and plasma reagents in the thromboplastin generation test (Fig. 33-6, page 1059). Normal levels of factor XII range from as low as 30%<sup>189</sup> to as high as 225%<sup>324</sup> of standard normal plasma. As with factor XI, accurate assays of factor XII depend on a source of fresh substrate plasma from a deficient patient. Laboratory demonstration of the heterozygous state is difficult.<sup>79</sup> The mean level of factor XII in the plasma of carriers is approximately 50% of normal, but observed values were distributed in a bimodal manner, an observation interpreted to suggest the presence of multiple abnormal alleles.<sup>205,406</sup>

The marked discrepancy between in vitro evidence of grossly abnormal blood coagulation and the absence of hemorrhagic manifestations in factor XII deficiency poses fundamental questions regarding the role of coagulation in hemostasis and the significance of laboratory measurements of coagulation. These questions cannot be answered at the present time, although various hypotheses have been formulated; these are discussed on page 438. For practical purposes, factor XII deficiency remains a laboratory curiosity.

## von Willebrand's Disease

The confusion that has surrounded the pathogenesis of von Willebrand's disease is apparent from the many names that have been applied to this disorder. These designations include *angiohemophilia*, *vascular hemophilia*, *pseudohemophilia*, *constitutional thrombopathy*, and "idiopathic" prolonged bleeding time. The disorder was first recognized by von Willebrand in a study of the inhabitants of the Åland islands, and is characterized by a prolonged bleeding time, mild to moderate

factor VIII deficiency, and mild mucocutaneous hemorrhage.<sup>63,223</sup>

## Genetics

There is now general agreement that von Willebrand's disease usually is inherited as an incompletely dominant autosomal trait.<sup>194</sup> The expressivity of this genetic abnormality is, however, highly variable, even among members of a single kindred.<sup>132-234</sup> A family history atypical of an autosomal dominant trait is not uncommonly obtained in von Willebrand's disease, and, in some kindreds, the disorder appeared to be transmitted on the X chromosome.<sup>173</sup> It is not improbable that several genetic abnormalities may underlie this disorder.<sup>63,401a</sup>

## Pathophysiology

### Prolonged Bleeding Time

Prior to the present decade it was thought that the abnormality in primary hemostasis was the result of either a primary vascular defect<sup>238</sup> or an intrinsic abnormality of the platelets.<sup>422</sup> Neither of these hypotheses has found significant support.

Most studies of platelet function have yielded normal results in patients with von Willebrand's disease. These have included measurements of platelet adhesion to collagen, the subsequent release reaction, and aggregation induced by ADP, collagen, and other substances.<sup>223-421,422</sup> Furthermore, transfusion of normal platelets does not shorten the bleeding time in this disorder.<sup>271</sup> Deficient platelet adhesion to the endothelial basement membrane,<sup>201,395a</sup> certain ultrastructural abnormalities of the platelets,<sup>64</sup> and a paradoxical increase in the optical density of platelet-rich plasma mixed with low concentrations of ADP<sup>223,402</sup> have been reported in a few patients. The significance of these observations remains uncertain.

More consistently, two abnormalities have been demonstrated; ie, platelets of affected persons are not retained normally in glass-

bead columns, and those of some patients do not aggregate in the presence of the drug ristocetin.<sup>181</sup> There is good evidence that these abnormalities and the prolonged bleeding time are due to the deficiency of a humoral factor, which, for lack of a better name, is usually termed the "anti-bleeding factor." This factor appears to be an antigenically competent fragment or subunit of the factor VIII molecule (*factor VIII related antigen* or CRM). Materials rich in this substance correct in vitro abnormalities in platelet retention<sup>60,256,422a</sup> and ristocetin-induced platelet aggregation<sup>422b,422c</sup> in the blood of patients with von Willebrand's disease. Plasma levels of factor VIII related antigen are unrelated to the levels of coagulant factor VIII,<sup>422b</sup> since similar results are obtained both in vivo and in vitro with "factor VIII" that has been concentrated or purified from hemophilic plasma.<sup>30,254</sup> However, a consistent correlation between factor VIII levels and the bleeding time has not been clearly established in all cases.<sup>329,329a</sup>

### Factor VIII Deficiency

In most patients with von Willebrand's disease the infusion of normal plasma produces a gradual and sustained rise in the coagulant factor VIII levels of the recipient, which reach a plateau 6 to 20 hours later and may persist for 48 to 72 hours<sup>272</sup> (Fig. 37-4). The factor VIII levels attained cannot be attributed to factor VIII present in the infused plasma, since comparable results are obtained with plasma deficient in factor VIII and with normal serum. In contrast, in patients with hemophilia A, the in vivo effect of a comparable amount of normal plasma is maximal immediately after infusion, but rapidly diminishes at a rate consistent with the short in vivo survival time of factor VIII (Fig. 37-4). This phenomenon, termed "*new factor VIII synthesis*" or "*in vivo complementation*," is presumably the result of the administration of a humoral factor ("*factor VIII-inducing substance*")<sup>263,362</sup> The phenomenon is not reciprocal; ie, the infusion of



plasma from a patient with von Willebrand's disease into a hemophiliac does not produce new factor VIII synthesis.<sup>154</sup> The factor VIII "newly synthesized" in transfused patients with von Willebrand's disease appears to differ immunologically from both the factor VIII present prior to transfusion, and that present in normal subjects.<sup>30,305a</sup>

The phenomenon of new factor VIII synthesis provides clear evidence that at least two genetic loci are involved in the regulation of factor VIII biosynthesis, i.e., (1) the locus on the X chromosome which is abnormal in classical hemophilia and codes the synthesis of normal amounts of functionally defective factor VIII; and (2) a locus on an autosome, which, being abnormal in von Willebrand's disease, results in deficient factor VIII synthesis. The possible identity of, or relationship between, the "anti-bleeding factor" and the "factor VIII-inducing substance" is unclear. In the few kindreds in whom adequate data are available, persons homozygous for the autosomal gene appear to have lower factor VIII levels than do the heterozygotes.<sup>153</sup> They also have more severe bleeding manifestations, and synthesize less "new" factor VIII following plasma infusions. These observations favor the view that the autosomal gene is concerned with production of either a subunit or a precursor of factor VIII<sup>153</sup> and is not merely a regulatory gene.<sup>153</sup>

An immunologic study of 77 patients with von Willebrand's disease demonstrated two distinct groups of patients.<sup>176</sup> In one group, comprised of approximately 75% of the patients, the amount of immunologically reactive factor VIII was proportional to the concentration of functional factor VIII.<sup>176</sup> The factor VIII deficiency in these patients thus is due to deficient biosynthesis of this protein, in contrast to the usual situation in hemophilia A.<sup>432</sup> In another group, consisting of approximately 25% of the patients, factor VIII related antigen was present in normal amounts, but appeared to be qualitatively abnormal.<sup>211a,393a</sup> In these patients, the disorder appeared to be inherited as an X-linked recessive trait, platelet retention as deter-

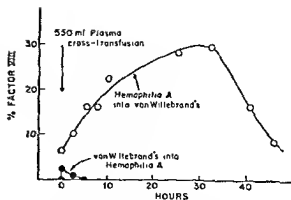


Fig 37-4. The phenomenon of "new" factor VIII synthesis. Cross transfusion of plasma from patients with classical hemophilia and von Willebrand's disease. The open circles represent the changes in the plasma factor VIII levels of a patient with von Willebrand's disease, following the infusion of 550 ml of plasma from a patient with severe hemophilia A. A significant and sustained increase in the factor VIII levels of the recipient was observed even though no active factor VIII was present in the infused plasma. The solid circles represent the effects of infusing 550 ml of plasma from a patient with von Willebrand's disease into a patient with severe hemophilia A. The factor VIII level in the infused plasma was 15% of normal. Note the slight and transitory effects. (From Shulman et al.,<sup>352</sup> courtesy of the authors and the *Annals of Internal Medicine*.)

mined by the Salzman method was normal, and the phenomenon of "new factor VIII synthesis" could not be demonstrated.<sup>176</sup>

Studies of ristocetin-induced platelet aggregation also suggested the presence of three different groups of patients with von Willebrand's disease.<sup>123a</sup>

Following transfusion of factor VIII into patients with hemophilia A, antigenically competent CRM persists in the circulation long after functionally active factor VIII has disappeared.<sup>26</sup> In von Willebrand's disease, essentially the reverse is found; i.e., antigenically recognizable factor VIII disappears rapidly, whereas coagulant activity remains for 24 to 48 hours.<sup>30</sup> These observations, together with the *in vitro* experiments discussed above, suggest that the major antigenic determinant of factor VIII is located on a portion of the molecule or molecular system that is dissociable, both *in vivo* and *in vitro*, from that portion which bears the site that

functions in coagulation; and, further, that in hemophilia A these two subunits or fragments are synthesized and metabolized in a manner distinctly different from that characteristic of von Willebrand's disease.<sup>27,30,328,372</sup>

### Incidence

Accurate figures concerning the incidence of von Willebrand's disease are lacking, owing to the inadequacy of available diagnostic criteria and the frequency of unrecognized mild or partial forms of the disorder. In many areas this disorder is second only to hemophilia A in frequency, and in some it appears to be the most common of all the hereditary coagulation disorders.

### Clinical Picture

The bleeding manifestations in von Willebrand's disease are consistent with the "hybrid" nature of the disorder. Thus, although the clinical picture is dominated by cutaneous and mucosal bleeding, hemarthroses<sup>105a</sup> and dissecting intramuscular hematomas may develop in the severely affected patient. As in mild classical hemophilia, serious hemorrhage due to traumatic injuries or following surgical procedures is a significant hazard.<sup>223</sup> Petechiae are rare, but gastrointestinal bleeding, epistaxis, and menorrhagia are particularly common. The bleeding manifestations in the usual patient with von Willebrand's disease are relatively mild, however, and patients with the partial forms may be virtually asymptomatic. It has been suggested that the disorder may decrease in severity with advancing age.<sup>223</sup> It definitely becomes milder during pregnancy, when the factor VIII level rises significantly.<sup>27</sup>

### Laboratory Diagnosis

Criteria for the laboratory diagnosis of von Willebrand's disease are, as yet, unsatisfactory.<sup>63 223,422</sup> Of the simpler tests (Table 37-4), both the bleeding time and the PTT may be abnormal. The abnormality in the PTT reflects a slight to moderate reduction

in factor VIII. Plasma factor VIII levels vary greatly. In most patients they range from 5 to 15% of normal,<sup>223</sup> but values up to 30% are not uncommon; rarely, they may be as low as 2 to 3% of normal. The coagulation time and the results of the prothrombin consumption test are normal, unless the factor VIII deficiency is unusually severe.

The Ivy bleeding time, or technical modifications thereof (page 1049), may be more sensitive than the Duke (earlobe) method in detecting the abnormality characteristic of von Willebrand's disease.<sup>422</sup> A method that attempts to quantify both the amount of blood lost and the duration of bleeding may be even more sensitive.<sup>390</sup>

Most methods for the determination of platelet adhesiveness to glass actually measure the retention of platelets within glass-bead columns (platelet retention). Among the numerous methods that have been described (page 1053),<sup>164,390,422</sup> the method of Salzman<sup>117</sup> is the most widely used, and in some hands has provided a valuable ancillary diagnostic criterion for von Willebrand's disease. Deficient retention has been demonstrated in from 80% to as high as 100% of cases,<sup>223</sup> and in many instances this test gave abnormal findings when the bleeding time and factor VIII levels were normal or equivocal. Other investigators<sup>4,164</sup> have obtained less consistent results. A major criticism of the technique has been the unusually wide range of results that may be obtained in the normal population.<sup>422</sup> The consistency of the method may be improved by several technical modifications,<sup>165</sup> and more reproducible results may be obtained with related methods employing heparinized platelet-rich plasma. The latter methods, however, are greatly affected by *in vitro* platelet manipulation.<sup>433</sup>

In mildly affected patients, the administration of small doses of aspirin appears to prolong the earlobe bleeding time (Duke's method). This phenomenon, the so-called *aspirin tolerance test*,<sup>312</sup> has been proposed as an adjunct for the diagnosis of von Willebrand's disease. The specificity of the technique may be seriously questioned<sup>20,422</sup> (page 1021). *Tests of ristocetin-induced platelet ag-*

gregation may be the most sensitive method of detecting mild von Willebrand's disease,<sup>422b,422c</sup> but have not as yet been thoroughly evaluated.

In von Willebrand's disease, one or more of the several abnormalities detected by laboratory means is frequently lacking, and, when present, may fluctuate from time to time.<sup>63</sup> In some patients, this may be the result of exogenous factors, eg, pregnancy, oral contraceptives, exercise,<sup>192</sup> or trauma, all of which have been shown to increase factor VIII levels in this disorder.<sup>117</sup> In many other patients, however, the cause is obscure. Even with the more elaborate confirmatory tests now available the diagnosis of this disorder may be difficult and may require repeated observations over a period of time.

## Miscellaneous Hereditary Coagulation Disorders

Several hemorrhagic disorders,<sup>293</sup> in addition to those described above, have been attributed to hereditary deficiencies of coagulation factors.<sup>78,85,88,342,353</sup> In most cases, their status as specific coagulation disorders is uncertain. Deficiency of *Carr factor*<sup>83</sup> refers to a mild hemorrhagic diathesis that affected several members of a single kindred. The disorder was present in both men and women. *Fletcher factor deficiency*<sup>166,167</sup> is a disorder characterized by abnormally slow contact activation. The PTT is normalized by prolonged incubation in systems containing particulate silicate.<sup>1</sup> Like Factor XII deficiency it is not associated with abnormal bleeding. It has been shown to be due to deficiency of prekallikrein.<sup>429a</sup> The genetics of both of these disorders are unclear.

The *Dyma abnormality* refers to a mild lifelong hemorrhagic diathesis that is inherited as an autosomal recessive trait. It has been described in only a single kindred.<sup>293</sup>

Of unusual interest are reports concerning the presence of combined deficiencies of two or more coagulation factors (Table 37-2). The most common of these appear to be deficiency of factors V and VIII,<sup>352,370a</sup> deficiency of factors VIII and IX,<sup>419,341,368</sup> and

combined deficiency of various vitamin K-dependent factors.<sup>134,220,284,411</sup> Several other combined coagulation defects have been described<sup>133,274,283,350,352</sup> (Table 37-2). Genetic details are lacking in most reports, and some of the reports are not entirely convincing.<sup>150</sup>

## Hereditary Coagulation Disorders in Lower Animals

Hemophilia A has been found in several breeds of dogs, and differs from the human disease in that cross-reacting material is not present in the plasma.<sup>420</sup> Both a clinically severe<sup>71,151</sup> form in Irish setters and a mild form in beagles<sup>76</sup> have been described. Hemophilia A also has been reported in a horse.<sup>279</sup> A bleeding disorder in swine closely resembles von Willebrand's disease in man, but its mode of inheritance is autosomal recessive rather than dominant.<sup>74</sup> In contrast to the human disorder, no unusual bleeding has been observed in canine factor VII deficiency<sup>264</sup> (Table 37-2).

## Therapy of the Hereditary Coagulation Disorders

Various styptics, drugs, diets, and hormones have periodically been advocated for the treatment of the hereditary coagulation disorders. These include "ovarian substance,"<sup>49,382</sup> oral contraceptives,<sup>22a</sup> vitamin K, rutin, bioflavonoids,<sup>811</sup> and peanuts or extracts thereof,<sup>196,405,410</sup> as well as splenic transplantation<sup>167a</sup> (page 417). None of these remedies is of proven value. Topical hemostatics may be of temporary value in small injuries, and certain other measures, to be discussed below, may prove to have some adjunctive value under specific circumstances, eg, hypnosis, corticosteroids,<sup>23</sup> inhibitors of fibrinolysis.<sup>151</sup> However, the only firmly established mode of treatment for the hereditary coagulation disorders is replacement therapy, ie, the intravenous administration of the required factor in the form of blood or blood products derived from normal persons or animals.<sup>55,323,337,339,359</sup>

Table 37.5. Biodynamic Properties of Coagulation Factors of Concern in Replacement Therapy

Disorder	Hemostatic Level (% of normal) (1)	Initial <i>In Vivo</i> Recovery (% of Infused Material) (2)	In Vivo "Survival" of Infused Coagulation Factors	
			First Phase Diffusion "Half- Life" (Hours) (3)	Second Phase Biologic "Half- Life" (Hours) (4)
Hemophilia A (factor VIII deficiency)	25-30	50-80	4-6	12
Hemophilia B (factor IX deficiency)	15-25	25-50	2-3	24
Fibrinogen deficiency	100 mg/dl	50	12	77-106
Prothrombin deficiency	720-40	50-100	8	72-96
Factor V deficiency	15-25	750-100	.	712-38
Factor VII deficiency	5-10	7100	0.5	5
Factor X deficiency	10-20	50-100	2-9	24-60
Factor XI deficiency	710	~100	.	748-84
Factor XIII deficiency	2-3	50-100	.	72-96

Values indicated for hemostatic levels represent the upper limits of published figures

Question marks indicate insufficient data or significant disagreement among published figures

\*In most patients survival curve is monophasic and lacks an initial component

### Replacement Therapy

The objective of replacement therapy is to obtain a concentration of the required factor in the bleeding site such that coagulation may become hemostatically effective.<sup>239</sup> Its achievement involves a consideration of several biodynamic properties of the various coagulation factors (Table 37-5), a general knowledge of the available therapeutic materials, and clinical assessment of the severity of the hemorrhagic manifestations.

### Hemostatic Levels

The hemostatic level may be defined as the lowest plasma concentration of a given coagulation factor that is required for normal hemostasis (Table 37-5, column 1). This value has been determined by purely empirical means, i.e., by measurement of the blood levels of the deficient factor at which bleeding appeared to stop in patients with one of the hereditary coagulation disorders during the course of replacement therapy. Needless to say, such estimates are very inaccurate. In patients with hemophilia A, the hemostatic level of factor VIII is approximately 25 to

30% of normal<sup>239</sup>; in those with hemophilia B, values from 15 to 25% of normal have been reported. Plasma levels of factor XIII as low as 2 to 3% of normal are adequate for normal hemostasis.<sup>359</sup>

### In Vivo Recovery and Survival of Infused Coagulation Factors

When a coagulation factor is infused intravenously into a recipient deficient in that factor, the levels present in the circulation after intravascular mixing are significantly lower than those that would be expected merely from dilution in the recipient's plasma. That this initial *in vivo* recovery of infused coagulation factors (Table 37-5, column 2) is usually less than 100% presumably is due to loss of these proteins into intravascular spaces. The adsorption of coagulation factors by platelets, various cells, vascular surfaces, and, in the case of factor VIII, "sequestration" in the spleen also may be involved. The initial recovery of infused coagulation factors is difficult to quantify, and ranges from nearly 100% recovery of factor XI to as low as 30%<sup>131</sup> of factor IX.

After *in vivo* mixing is complete, the activity of most coagulation factors in the plasma declines in a biphasic manner; i.e., an initial rapid loss of activity is followed by a more gradual decline. The *first or rapid phase* presumably is the result of diffusion into extravascular pools.<sup>359</sup> This "diffusion half-life" (Table 37-5, column 3) ranges from minutes for factor VII, to several hours for factor VIII. In general, it is the rapidity of this first phase, together with the initial *in vivo* recovery of the particular factor, that determines the necessity for and the size of the preliminary or "loading" dose of therapeutic material.

The *second or slow phase* of the survival curve presumably is the result of degradation and reflects the true biologic half-life of the infused factor (Table 37-5, column 4). This parameter, together with the hemostatic level for the factor of concern, is the main determinant of the frequency of administration and the size of the maintenance dose of therapeutic material. For example, approximately 80% of factor VIII infused in the form of *fresh frozen plasma is initially recovered in the circulation; its initial ("diffusion") and subsequent ("biologic") half-lives are approximately 6 and 12 hours, respectively.*<sup>359</sup> Thus, in the treatment of patients with hemophilia A, a modest loading dose is usually employed, and maintenance doses are administered every 8 to 12 hours. In patients with hemophilia B, the initial recovery of factor IX is 50% or less<sup>11</sup>; its initial and subsequent half-lives are approximately 3 and 24 hours, respectively. Hence a very large loading dose is essential, but only small and infrequent maintenance doses are required.

After a large loading dose, or after several courses of therapeutic material have been administered, the "survival curves" of infused coagulation factors become nearly monophasic, presumably because extravascular spaces and the other mechanisms that remove infused therapeutic material from the circulation have become "saturated." With some factors, such monophasic curves are characteristic, e.g., factor XI, and, in some patients, factor V.<sup>418</sup>

## Therapeutic Materials

Prior to 1960, plasma was the only agent generally available for the treatment of the hereditary coagulation disorders. Several concentrated blood products are now available for this purpose. The activity of the various coagulation factors in such concentrates is expressed in terms of units. One such unit is defined as that activity present in 1 ml of fresh plasma from normal male donors. The concentration of all coagulation factors in native plasma is thus 1 unit/ml; levels in bank plasma are 0.8 U/ml because of the dilution with anticoagulant. The potency or extent of purification of various preparations of coagulation factors usually is expressed in terms of the ratio of coagulant activity (per unit weight of protein) in the concentrate to that in plasma.<sup>337</sup>

## Plasma

Rapid losses of factors V and VIII are observed in plasma anticoagulated with EDTA or oxalate. These factors also are lost in stored citrated plasma, but at a slower and more variable rate.<sup>318,378</sup> Consequently fresh or frozen plasma is preferable for treatment of patients with hemophilia A and of those with factor V deficiency. Stored plasma, outdated plasma, and supernatant plasma after removal of the cryoprecipitate contain essentially normal levels of prothrombin, fibrinogen, and factors VII, IX, X, XI, and XIII. Such plasma is suitable for replacement therapy in persons with disorders due to deficiency or abnormality of these substances.

Both the rate of administration, and the total dose of plasma that can be given are limited by the possibility of acute or chronic circulatory overload. The volume expansion resulting from even moderate doses of plasma also limits the blood levels that can be attained. As a consequence, therapy with plasma alone cannot be expected to increase the levels of a deficient factor more than 20% above baseline values.<sup>337</sup> When plasma is the only therapeutic agent available, plasmapheresis may be of adjunctive value.<sup>294</sup>

### *Purified or Concentrated Coagulation Factors*

**CRYOPRECIPITATES.** A major advance in the therapy of hemophilia A was the demonstration by Pool and her coworkers that cold insoluble material obtained from plasma contains high concentrations of factor VIII and fibrinogen.<sup>170-303</sup> This cryoprecipitate, which for many years was discarded during "clarification" of plasma, is prepared by slowly thawing rapidly frozen plasma at 2 to 4°C and then harvesting the precipitate by centrifugation. Cryoprecipitate prepared from 200 ml of fresh plasma contains from 50 to 120 units of factor VIII, approximately 250 mg of fibrinogen, and therapeutically useful amounts of factor XIII, as well as the "factor VIII-inducing substance" that is deficient in von Willebrand's disease. Cryoprecipitation provides 7 to 20 times purification of factor VIII with respect to plasma, and can be carried out by means of a closed double-bag system even in small routine blood banks without special equipment. Because of its simplicity it has proved to be a particularly valuable method of concentrating factor VIII.

**PURIFIED FACTOR VIII.** Among the many methods that have been developed to purify factor VIII (page 417), several have proved suitable for the large-scale production of concentrates of the human factor for therapeutic use. The resulting fractions represent from 10- to as high as 400-fold purification of factor VIII with respect to plasma. They are sufficiently potent to attain *in vivo* factor VIII levels as high as 100% in hemophilic patients without significant expansion of the plasma volume.<sup>12,36,55,338</sup> Most of these preparations of factor VIII are standardized and are provided with a predetermined assay of their potency, which is an advantage in the treatment of major bleeding. High-potency concentrates are relatively more convenient to administer than cryoprecipitates or less concentrated preparations of factor VIII. For example, therapeutic doses may be administered by a syringe, which may be a convenience in various home-care or prophylactic

programs to be discussed below. With these important exceptions, however, it is probable that highly concentrated factor VIII offers no significant advantages over cryoprecipitates, except under unusual circumstances in which very high blood levels of this factor are required. A considerable risk of hepatitis attends the use of most concentrated preparations,<sup>415</sup> since they are prepared from large plasma pools. This may be less of a problem with preparations of high purity.<sup>199</sup> The initial recovery of active factor VIII in the circulation appears to vary with the particular preparation,<sup>110,338,371</sup> a minor disadvantage requiring slight adjustment of the dosage for each preparation. The cost per unit of factor VIII is approximately the same whether concentrates or cryoprecipitates are used.

Factor VIII preparations of both high and intermediate potency are available through the American National Red Cross<sup>199</sup> and from commercial sources in the United States<sup>87</sup> and elsewhere.<sup>86</sup> The biochemical methods used to prepare these purified materials, and details concerning their properties, are beyond the scope of this book. Detailed information has been summarized elsewhere.<sup>31,187,199,211,248,366</sup>

**FACTOR VIII OF ANIMAL ORIGIN.** Factor VIII has been purified as much as 1000-fold from porcine or bovine plasma.<sup>31,239</sup> Such preparations are of proven clinical effectiveness, and were particularly valuable in the treatment of patients having major hemorrhage and during surgical procedures when human factor VIII was in limited supply. However, available preparations are antigenic, a drawback that limits their use to a single therapeutic course. Mild thrombocytopenia and other adverse reactions also have been described in the recipient. Heterologous factor VIII apparently is less reactive with acquired antibodies to factor VIII ("circulating anticoagulants") than is the homologous protein,<sup>360</sup> a property that may prove advantageous in the treatment of bleeding due to such antibodies (page 1208). Animal factor VIII is not as yet commercially available in the United States.

"PROTHROMBIN COMPLEX." The four vitamin K-dependent factors (prothrombin and factors VII, IX, and X) are avidly adsorbed by aluminum hydroxide or barium sulfate, and can be readily concentrated by elution therefrom. This simple procedure is the starting point for several methods of preparing therapeutically useful concentrates of these proteins.<sup>110,113,377,397</sup> A preparation containing 60 times the plasma concentrations of all four of these factors is now commercially available.<sup>93</sup> Variable amounts of factor XI also are present in these preparations. Preliminary data would suggest that this concentrate may greatly improve the efficacy of replacement therapy in patients with deficiencies of these factors.<sup>116,173</sup> As with purified factor VIII, this concentrate may be contaminated with the hepatitis virus.

### "Major" and "Minor" Bleeding

For purposes of replacement therapy, the various bleeding manifestations commonly encountered in the hereditary coagulation disorders may be divided into major and minor categories. Although this distinction is relative and somewhat artificial, it has proved helpful clinically.

Manifestations falling into the "minor" category include bleeding associated with uncomplicated hemarthrosis, symptomatic hematomas in noncritical areas and very minor traumatic injuries, as well as such therapeutic procedures as arthrocentesis, dressing changes, and removal of stitches or drains. Small cuts and scratches, superficial ecchymoses, and small hematomas ordinarily require no replacement therapy. Minor bleeding will usually respond to regimens that produce a hemostatic level once or twice in a 24-hour period.

Major bleeding is by definition life-threatening, and includes hematomas in critical locations and bleeding due to traumatic injuries, particularly those in which there is significant external blood loss, and to surgical procedures, including tonsillectomy and the extraction of even a single molar tooth. In the treatment of major bleeding, the *in vivo*

levels of the deficient factor should be continuously maintained above the hemostatic level. In bleeding of a particularly critical nature such as intracranial hemorrhage or for the prevention of excessive bleeding during neurosurgery, it is probable that even higher levels should be maintained.

### Regimens

Although the various considerations that have been discussed in the foregoing paragraphs provide the scientific basis for replacement therapy in patients with hereditary coagulation disorders, it must be recognized that variations in the responses of individual patients are common, and that, to a certain extent, treatment of persons with these disorders must be individualized. This is particularly true for therapy of patients with the very rare disorders, in whom the hemostatic levels and the biodynamic properties of the deficient factors are poorly defined. Thus, the generalizations discussed below, as well as the regimens summarized in Tables 37-6 and 37-7, should be regarded only as a "first approximation" to the therapy of patients with these disorders.

### Hemophilia A

Although it is effective in *minor bleeding*, plasma is now seldom used in the treatment of patients with hemophilia A. Cryoprecipitates are now widely available and are the most generally useful therapeutic material.<sup>367</sup> Both more and less factor VIII than indicated in Table 37-6 have been recommended.<sup>66,94</sup> For most minor bleeding manifestations, treatment for a minimum of three days is required. Regimens employing a single dose of 20 to 30 U factor VIII/kg have been advocated for the management of hemarthrosis, but have not proved uniformly effective.<sup>216</sup> For the removal of sutures, drains and dressing changes, a single dose of 10 to 15 U/kg of factor VIII usually will suffice. In the treatment of minor bleeding, loading doses are not required, and laboratory monitoring of *in vivo* factor VIII levels is unnecessary.

Table 37-6. Replacement Therapy in Hemophilia A and Hemophilia B

Minor Bleeding			Major Bleeding	
	Therapeutic Material	Loading Dose	Maintenance Dose	
<i>Uncomplicated hemarthroses, hematomas in noncritical areas, hematoma dressing changes,* arthrocentesis,* removal of sutures and drains*</i>				
HEMOPHILIA A (Factor VIII deficiency)	Cryoprecipitate	Not required	1.25-1.75 bags/10 kg every 12 hrs for 2-4 days	3.5 bags/10 kg
	Purified factor VIII†	Not required	10-15 U/kg every 12 hrs for 2-4 days	1.75 bags/10 kg every 8 hours for 1-2 days, every 12 hours thereafter
	Fresh or fresh frozen citrated plasma‡	Not required	10-15 ml/kg every 12 hrs for 2-4 days	10-15 U/kg every 8 hours for 1-2 days; every 12 hours thereafter
HEMOPHILIA B (Factor IX deficiency)	Citrated plasma §	30 ml/kg	7 ml/kg every 12 hrs for 2-4 days	15 ml/kg every 8 hours for 1-2 days, every 12 hours thereafter
	Purified "Prothrombin Complex" †	30 U/kg	10 U/kg every 12 hrs for 2-4 days	7 ml/kg every 12 hours
				10 U/kg every 12 hours

\* Single dose of 15 U/kg or equivalent amounts of concentrated material are usually sufficient

† Initial in vivo recovery of active factor varies somewhat depending on particular preparation

‡ Recommended only if other material is not available

§ Stored, outdated plasma, or plasma after removal of cryoprecipitate is adequate



In the therapy of *major bleeding* in patients with hemophilia A, an initial loading dose of factor VIII should always be administered, and sufficient factor VIII must be given frequently enough to assure that the blood level does not fall below 25% for any length of time.<sup>245</sup> Maintenance doses usually are given every 8 or 12 hours, although some saving of therapeutic material is accomplished by more frequent administration. Regimens employing the continuous infusion of factor VIII have been described.<sup>249</sup> Although theoretically sound, such regimens usually are inconvenient and time-consuming. It should be pointed out that the treatment of post-surgical or major traumatic hemorrhage in patients with mild hemophilia A requires very nearly as much therapeutic material as is needed for the severely affected patient.

There is no general agreement concerning the duration of replacement therapy when major bleeding occurs. Therapy has been continued for 10 to 14 days in most hemophiliacs in whom major surgical procedures were carried out successfully. Many investigators administer the full doses indicated in Table 37-6 for 10 days, followed by half doses for four days. The necessity for such protracted therapy has been questioned.<sup>136</sup> Therapy for five to seven days usually suffices following tooth extractions.

Determination in the laboratory of the *in vivo* levels of factor VIII is highly desirable in the treatment of patients having major hemorrhage. The peak level of factor VIII 10 minutes after an infusion of therapeutic material should be approximately 75% if the level is not to fall below 25% on a 12-hour schedule. The use of the PTT or some other one-stage screening test to replace a specific factor VIII assay cannot be recommended, since the results of these tests may be normal at hazardously low levels of factor VIII.<sup>359</sup>

### *Hemophilia B*

Most of the comments concerning the therapy of patients with hemophilia A apply to the treatment of those with hemophilia B. Because of the low initial *in vivo* recovery and the rapid initial disappearance of factor

IX from the circulation, loading doses are recommended even when minor bleeding occurs. In patients with hemophilia B, it is often difficult to attain hemostatic levels with plasma alone,<sup>337</sup> and in general concentrated material is recommended when major hemorrhage is present.<sup>173</sup> If plasma is the only agent available, very large amounts (60 ml/kg) are infused at the maximum tolerated rate.<sup>359</sup> This is followed by smaller doses at 12-hour intervals (Table 37-6).

### *von Willebrand's Disease*

In the usual patient with von Willebrand's disease, bleeding manifestations are mild, and replacement therapy is seldom required. Because of the phenomenon of "new factor VIII synthesis" (page 1181), the daily administration of small amounts of plasma will usually produce hemostatic levels of factor VIII (Table 37-7).<sup>42,359</sup> In the severely affected patient with marked factor VIII deficiency, relatively little "new" factor VIII is synthesized endogenously, and such patients should be managed much like those with hemophilia A<sup>359</sup> (Table 37-7). In some patients with von Willebrand's disease, the severity of bleeding does not appear to be related to the factor VIII level, and presumably is the result of the abnormality in primary hemostasis. There is no effective therapy for this abnormality, and chronic gastrointestinal hemorrhage and menorrhagia, in particular, may be virtually intractable. Although various therapeutic materials will shorten the bleeding time transiently,<sup>42,50,422</sup> this test should not be employed as a monitor of the efficacy of treatment in this disorder.<sup>42</sup>

In the preoperative preparation of patients with von Willebrand's disease, replacement therapy should begin 24 hours before the operation in order that "newly" synthesized factor VIII may raise the factor VIII level endogenously. Some preparations of purified factor VIII contain little "factor VIII-inducing substance," or "anti-bleeding" factor<sup>295</sup>; for this reason, cryoprecipitates or plasma is preferred for the treatment of patients with von Willebrand's disease.

Table 37-7. Replacement Therapy in Miscellaneous Hereditary Coagulation Disorders

Disorder	Therapeutic Material	Loading Dose	Maintenance Dose	References
von Willebrand's Disease	Fresh or fresh frozen plasma*	Not required	10 ml/kg daily	42, 63, 223, 359, 362
	Cryoprecipitate	Not required	1 bag/10 kg daily	
Fibrinogen deficiency	Cryoprecipitate	4 bags/10 kg	1 bag/10 kg every other day	359
	Purified fibrinogen†	100 mg/kg	20 mg/kg every other day	
Prothrombin deficiency	Stored plasma	15 ml/kg	5-10 ml/kg daily	38, 110, 359
	Purified prothrombin complex	20 U/kg	10 U/kg daily	
Factor V deficiency	Fresh or fresh frozen plasma	20 ml/kg	10 ml/kg every 12 hours	251, 345, 352, 359, 418
Factor VII deficiency	Stored plasma‡	10 ml/kg	5 ml/kg every 6-24 hours	110, 172, 244, 359
	Purified prothrombin complex	10 U/kg	10 U/kg every 6-24 hours	
Factor X deficiency	Stored plasma‡	15 ml/kg	10 ml/kg daily	38, 110, 339, 359
	Purified prothrombin complex	15 U/kg	10 U/kg daily	
Factor XI deficiency	Stored plasma‡	10 ml/kg	5 ml/kg daily	177, 280, 343, 359
Factor XIII deficiency	Stored plasma	5 ml/kg every 1-2 weeks	Not required	113, 191, 359

\*Stored plasma contains adequate inducing factor but may be deficient in factor VIII

†Any purified factor VIII preparation or fibrinogen preparation is satisfactory in equivalent concentrations

‡Plasma after removal of cryoprecipitate is satisfactory

### Miscellaneous Disorders

Therapeutic regimens that have been recommended for the treatment of patients having any of the remaining hereditary coagulation disorders are summarized in Table 37-7, and details concerning treatment will be found in the references provided. In persons with factor XI deficiency, major bleeding is exceedingly rare, and small doses of plasma usually have been effective (Table 37-7). Patients with factor V deficiency also usually

respond well to fresh or fresh frozen plasma. The management of major bleeding due to deficiencies of factors VII and X and prothrombin has been greatly facilitated by the development of concentrates of the "prothrombin complex." Persons with factor XIII deficiency may be readily treated even with weekly administration of plasma; in some, such therapy appeared to induce a prolonged rise in *in vivo* levels suggesting "new synthesis" of this factor.<sup>160</sup> There is as yet little information concerning the treatment of patients with dysfibrinogenemia.

## Prophylactic Therapy

The regular administration of therapeutic material with the object of reducing and possibly eliminating spontaneous bleeding manifestations is a theoretically logical approach to the treatment of persons with the hereditary coagulation disorders.<sup>226</sup> Because of the development of concentrated preparations of factor VIII, such maintenance therapy has now become feasible in patients with hemophilia A. The administration of 1500 to 3000 U of factor VIII three to four times a week has significantly reduced the incidence of hemarthrosis.<sup>171, 403</sup> Trials employing smaller or less frequent administration of factor VIII have yielded less dramatic results.<sup>209, 276</sup> The practicality of routine maintenance therapy with such large amounts of factor VIII remains uncertain. Although the expense of these regimens is not a valid argument against their use,<sup>207</sup> the available supply of whole blood may ultimately be a limiting factor.

The concept of prophylactic therapy seems much more applicable to disorders in which the deficient factor has a long *in vivo* half-life. Prophylactic therapy is very effective in patients with factor XIII deficiency,<sup>71</sup> and encouraging results have been obtained in those with hemophilia B.<sup>173</sup> It has yet to be evaluated in the other hereditary coagulation disorders.

## Home-Treatment Programs

It is the general experience that replacement therapy in hemarthrosis is more effective, and subsequent joint damage is minimized, if replacement therapy is begun *immediately* following the onset of symptoms. The availability of factor VIII concentrates that are stable in home refrigerators has led to the development of various "early home-care" programs. Parents are trained to administer the material at the first sign of bleeding.<sup>173, 207, 316</sup> This can be recommended as the initial course of treatment. Individual factors will determine whether or not further treatment needs to be supervised by a physician.<sup>230</sup>

## Special Aspects of Treatment

### Dental Care

Special attention should be given to preventive dental care of patients with coagulation disorders, so as to minimize the complications, expense, and hazards of operative dental procedures. The extraction of even a single tooth requires replacement therapy. Multiple extractions may save time and expense but create a major bleeding hazard.<sup>359, 419</sup> Acrylic splints, orthodontic rubber bands, and other specialized dental techniques markedly reduce the chance of serious postoperative bleeding.<sup>419</sup> The suturing of bleeding tooth sockets following extractions, particularly of the third molar, should be avoided since it may lead to extension of bleeding into the neck. Mandibular block anesthesia has produced similar complications in some patients, but appears to be safe if performed expertly.<sup>419</sup>

It has been claimed that hypnosis<sup>236</sup> and inhibitors of fibrinolysis such as epsilon-aminocaproic acid (EACA) have adjunctive value in the prevention of bleeding that may follow operative dental procedures in patients with either hemophilia A or hemophilia B.<sup>128a, 148, 331, 337, 417</sup> On the basis of available data, these measures cannot be recommended to the exclusion of replacement therapy. Inhibitors of fibrinolysis have *no demonstrable* effect on spontaneous bleeding manifestations in patients with these disorders.<sup>26, 387</sup>

### Hemarthrosis

All patients with hemarthroses should receive adequate replacement therapy, since only in this manner can the permanent disability resulting from repeated bleeding into the joints be minimized.<sup>112, 200</sup> Pain usually is relieved promptly and is a reliable index of the therapeutic response. Supportive therapy includes immobilization and the administration of analgesics. A short course of corticosteroids was of adjunctive value in one series.<sup>216</sup> Although a time-honored procedure, the value of packing the joint in ice is uncertain.

*Arthrocentesis* usually is unnecessary, but may be of significant benefit when the joint is severely distended or when resolution of the hemarthrosis is delayed despite adequate replacement therapy. The effectiveness of this procedure has not been studied systematically, however. Large volumes of blood seldom can be aspirated, but even a small volume may relieve symptoms. If performed, arthrocentesis should be done immediately before the administration of a "covering" dose of factor VIII.<sup>91</sup>

Early but careful *physiotherapy* aimed at restoring full range of motion of the affected joint should be instituted as soon as the acute stage of hemarthrosis has resolved. More energetic physiotherapeutic techniques should be carried out only in conjunction with an adequate course of replacement therapy. Various orthopedic devices that provide additional support for chronically injured joints have proved useful in reducing the frequency of recurrent hemarthrosis, particularly in the knee and ankle joints, eg, Hessler braces, removable bivalve plaster casts.<sup>91</sup> *Synovectomy* has been recommended in the treatment of patients with chronically recurrent hemarthrosis in seriously damaged joints.<sup>386</sup>

### Miscellaneous

*Styptics* such as Gelfoam, Stypten, and bovine topical thrombin may arrest bleeding from readily accessible sites, if the overlying clots are carefully removed and the material is applied directly to the injured tissues.<sup>66</sup> *Epistaxis* often stops spontaneously and usually only conservative treatment is required. Intranasal tamponade, described on page 1149, has proved helpful in managing this complication. *Hematuria*, to the contrary, may be virtually intractable, and in some patients will not respond to even vigorous replacement therapy. In such patients, 20 to 40 mg prednisone, given daily for one week, may stop bleeding.<sup>3,149</sup> The mechanism of this therapeutic effect is obscure. EACA is contraindicated in the treatment of patients having hematuria, since it may produce intrarenal

obstruction with blood clots.<sup>381</sup> *Surgical procedures* in patients with hereditary coagulation disorders carry greater risk than in the general population,<sup>245</sup> even when optimal replacement therapy is administered, and should be avoided if at all possible. Treatment of bleeding in patients with *acquired antibodies* to the various factors is very difficult. This complication occurs most commonly in those with hemophilia A, and is discussed further on page 1208.

*Narcotics* should be used for pain relief only when absolutely necessary, because of the possibility of addiction. *Aspirin* is contraindicated since it may produce gastric erosions and gastrointestinal bleeding, and may also aggravate or provoke hemorrhagic episodes because of its inhibitory effects on platelet function<sup>201</sup> (page 1129).

Profound *psychologic and sociologic problems* often arise in families affected with the hereditary coagulation disorders and their management may constitute an important part of the overall care of the hemophilic patient.<sup>7</sup> Some useful guidelines for *psychiatric, genetic,<sup>212</sup> and occupational<sup>209</sup> counseling* of families affected with hereditary coagulation disorders have been published.<sup>209</sup>

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## Acquired Coagulation Disorders

Deficiencies of Vitamin K-Dependent Factors  
Hemorrhagic Disease of the Newborn  
Miscellaneous Causes of Vitamin K Deficiency  
Hepatic Disease  
Pathologic Inhibitors of Coagulation (the Circulating Anticoagulants)  
Specific Inhibitors  
Inhibitors in Lupus Erythematosus  
Miscellaneous  
Diffuse Intravascular Coagulation  
Fibrinogenolysis  
Miscellaneous

**A**BNORMALITIES in blood coagulation may complicate a large variety of disorders (Table 38-1). These acquired coagulation disorders are more complex than the hereditary forms. In contrast to hereditary disorders in which lack of a single factor is characteristic, in the acquired forms deficiency of several factors usually is found and thrombocytopenia, abnormalities in platelet function, abnormal inhibitors of coagulation, and vascular abnormalities are commonly present as well. Because of the "compound" nature of the hemostatic defect, the severity of bleeding correlates poorly with the results of laboratory tests in acquired coagulation disorders, and replacement therapy often is ineffective. With some notable exceptions, however, bleeding usually is less severe than in the hereditary forms and the clinical pic-

ture often is complicated by signs and symptoms of the underlying disease.

### Deficiencies of Vitamin K-Dependent Factors

Prothrombin and factors VII, IX, and X are synthesized in the liver by a process that is dependent on vitamin K (page 414). Deficiencies of these four factors may develop in a variety of disorders in which there is deficient intake or absorption of vitamin K, as well as in disorders that impair the biosynthetic capacity of the liver (Fig. 38-1). Anticoagulant drugs of the coumarin and indanedione groups antagonize the action of vitamin K and produce an identical coagulation abnormality (Chapter 39).

#### Hemorrhagic Disease of the Newborn (HDN)

Hemorrhagic disease of the newborn is the result of vitamin K deficiency in the neonate. Formerly a major cause of bleeding in the newborn,<sup>27</sup> this disorder is now uncommon because of the routine administration of vitamin K at birth.<sup>27</sup> However, it is still encountered in economically deprived populations.

#### Pathophysiology

There is a moderate but significant deficiency of the vitamin K-dependent coagula-

Table 38-1. The Acquired Coagulation Disorders

I Deficiencies of the vitamin K-dependent coagulation factors	
A	Hemorrhagic disease of the newborn
B	Biliary obstruction (gallstones, strictures, fistulas)
C	Malabsorption of vitamin K (sprue, idiopathic steatorrhea, celiac disease, ulcerative colitis, regional enteritis, gastrocolic fistulas, ascariis infestation)
D	Liver disease
E	Drugs
	1 Pharmacologic antagonists of vitamin K (coumarins, indanediones, diphenylhydantoin, salicylates)
	2 Those that alter gut flora (broad-spectrum antibiotics, sulfonamides)
F	Nutritional deficiency
II Inhibitors of coagulation (Circulating anticoagulants)	
A	Specific inhibitors (antibodies to fibrinogen factors V, VIII, IX, XI, XII, and XIII)
B	In systemic lupus erythematosus
C	Miscellaneous
III Accelerated destruction of coagulation factors	
A	Diffuse intravascular coagulation (DIC) (Table 38-3)
B	Fibrinogenolysis (DIC, liver disease, tumors following surgery)
IV Miscellaneous	
A	Liver disease
B	Disorders of the hematopoietic system (paraproteinemias, polycythemia vera, myelofibrosis, lymphomas, leukemia, others)
C	Others (following massive blood transfusion, uremia, amyloidosis, nephrotic syndrome, drugs such as L-asparaginase <sup>22</sup> )

tion factors in the normal newborn (page 420). The plasma levels of these factors normally fall even further during the first two to five days of life, rise again when the infant is seven to fourteen days of age, and attain normal adult levels at about three months of age.<sup>23</sup> This sequence of events normally does not produce bleeding, but in hemorrhagic disease of the newborn the initial fall is accentuated, and the secondary restoration is delayed and incomplete. As a consequence, coagulation abnormalities become marked and bleeding results.

The deficiency of the vitamin K-dependent coagulation factors that is present at birth, as

well as the slow rate at which adult levels are attained, presumably is the result of intrinsic "hepatic immaturity"; neither of these physiologic events is affected by vitamin K administration.<sup>23</sup> On the other hand, the "secondary" fall of these factors at age two to five days is prevented by vitamin K administration and is the result of a transitory "physiologic" deficiency of vitamin K. Factors that further diminish the amount of vitamin K available at this juncture, and those that further impair the synthetic capacity of the liver, predispose to hemorrhagic disease of the newborn. These are: (1) prematurity, (2) inadequate dietary intake, (3) delayed gut colonization by bacteria, and possibly (4) maternal deficiency of vitamin K and (5) various obstetric and perinatal complications.

*Prematurity* has frequently been associated with hemorrhagic disease of the newborn.<sup>1,2,20</sup> Levels of the vitamin K-dependent factors at birth are approximately proportional to gestational age and birth weight.<sup>5</sup> In premature infants, the "physiologic immaturity" of the liver is marked, and the response to the vitamin K that is present is subnormal.

Most factors associated with *deficient intake* of vitamin K also delay the *colonization of the gut* by bacteria. These include delayed feeding, breast feeding, vomiting, severe diarrhea, and antibiotics, including those present in mother's milk. Maternal milk and colostrum are poor sources of vitamin K,<sup>23</sup> and reliance on breast milk as the sole source of nutriment in the neonatal period is an important factor in many cases of hemorrhagic disease of the newborn.<sup>27</sup>

The importance of placental transfer of vitamin K during the antenatal period is uncertain, as is the contributory role of maternal deficiency of this vitamin. Traumatic delivery, especially if it is associated with prolonged hypoxia, and various other obstetric and perinatal complications may predispose to hemorrhagic disease, presumably by impairing hepatic function<sup>3</sup>; the evidence for the importance of these factors is largely circumstantial. Coumarin and indanedione

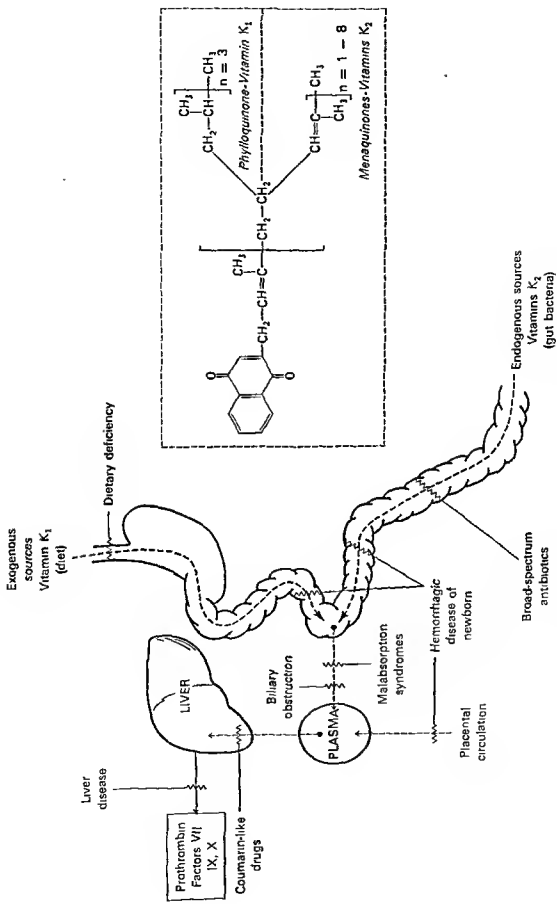


Fig. 38-1. Causes of deficiencies of the vitamin K-dependent coagulation factors. The origin, absorption, and transport of vitamin K under normal circumstances are illustrated by dashed lines. Processes leading to deficiency of this vitamin are indicated by solid lines. The structures of the K vitamins are shown in the inset.

drugs, as well as diphenylhydantoin,<sup>25a</sup> cross the placenta and may produce hemorrhagic disease in the newborn.<sup>10</sup>

### Clinical and Laboratory Features

Bleeding usually is severe,<sup>30</sup> and commonly first occurs on the second or third day of life.<sup>23</sup> The most common manifestations are melena (melena neonatorum), large cephalohematomas, and bleeding from the umbilical stump and following circumcision. Generalized ecchymoses, often without petechiae,<sup>3,23,30</sup> intracranial bleeding, and large intramuscular hemorrhages also may develop.

*Laboratory diagnosis* is relatively simple (Table 38-2), but the physiologic differences in the results of various coagulation tests in neonates as compared with those at a later age must be kept in mind (page 1067). In infants with hemorrhagic disease of the newborn the prothrombin time always is markedly prolonged and the PTT and the thrombin time also are prolonged; however, the results of these tests may also be abnormal in unaffected neonates (page 1067). The coagulation time of whole blood usually is prolonged in infants with hemorrhagic disease of the newborn, in contrast to most forms of vitamin K deficiency in adults. Specific assays reveal marked deficiencies of prothrombin and of factors VII, IX, and X. Factors V, VIII, and fibrinogen are present in normal amounts. The bleeding time and the platelet count usually are within normal limits.

It should not be assumed that bleeding in the neonate is invariably the result of vitamin K deficiency. In the *differential diagnosis of HDN*, virtually all causes of bleeding, particularly thrombocytopenia (page 1090) and diffuse intravascular coagulation (DIC), must be considered.<sup>7</sup> Various forms of DIC have been referred to by the somewhat confusing term "secondary hemorrhagic disease of the newborn."<sup>1,7,23</sup> The hereditary coagulation disorders (Chapter 37) also may produce serious hemorrhage in the neonatal period. Significant prolongation of the prothrombin time is not found in the most common hereditary disorders, eg, hemophilia A and hemo-

philia B. Umbilical bleeding and hemorrhage following circumcision are relatively less common in the hereditary coagulation disorders than in hemorrhagic disease of the newborn.

### Treatment

Vitamin K<sub>1</sub> (0.5 to 1.0 mg given intravenously) is dramatically effective in the treatment of hemorrhagic disease of the newborn. Shortening of the prothrombin time may be expected within six hours, and normal "neonatal" levels of the four vitamin K-dependent factors usually are attained within 24 hours. In severe cases, two or three doses of vitamin K<sub>1</sub> at four- to eight-hour intervals may be required, and the transfusion of fresh blood may be helpful.<sup>23</sup> Replacement therapy with plasma or concentrates of the vitamin K-dependent factors is quite effective (page 1187), but seldom is necessary. Premature infants usually respond poorly to vitamin K<sub>1</sub>. Larger (2 mg) or repeated (every four to eight hours) doses of vitamin K<sub>1</sub> may be required to counteract the effects of coumarin drugs.<sup>23</sup>

The administration of large doses of vitamin K may produce hemolysis, hyperbilirubinemia, and even kernicterus in the neonate. This appears to be a greater hazard with the synthetic derivatives than with vitamin K<sub>1</sub>, but even the latter may be dangerous in very large doses.<sup>23</sup>

Hemorrhagic disease of the newborn can be prevented by administration of vitamin K *to the mother prior to delivery*. However, most authorities recommend that vitamin K<sub>1</sub> (1 mg, parenterally; 2 mg, orally) be given to the infant. This procedure is now routine in most nurseries.

### Miscellaneous Causes of Vitamin K Deficiency

*Obstruction of the biliary tract*, either intrahepatic or extrahepatic, produces vitamin K deficiency because of the absence of bile salts in the gut. Complete obstruction may lead to severe coagulation abnormalities and bleeding within two to four weeks. This was a major obstacle to surgical procedures on the



biliary tract prior to the discovery of vitamin K.

Most *malabsorption syndromes* and various other chronic gastrointestinal disorders also may give rise to vitamin K deficiency, eg, celiac disease, cystic fibrosis,<sup>37</sup> sprue,<sup>21</sup> gastrocolic fistulas, ulcerative colitis, regional enteritis, extensive gut resections, protracted diarrhea of any cause,<sup>29</sup> ascaris infestations.<sup>3,18,34</sup> Severe abnormalities of coagulation with bleeding are less common in these disorders than in biliary obstruction, presumably because absorption of vitamin K is seldom completely deficient.

Because vitamin K normally is available from two independent sources (Fig. 38-1), neither *nutritional deficiency*<sup>29</sup> nor *gut sterilization* alone produces vitamin K deficiency of a degree that results in significant deficiencies of the vitamin K-dependent coagulation factors.<sup>8</sup> However, when broad-spectrum antibiotics or nonabsorbable sulfonamides are given to patients who are already nutritionally deficient, coagulation abnormalities and bleeding may result.<sup>12,15</sup>

There is indirect evidence that antibiotics, poor diet, or any of the above-mentioned disorders may predispose to coumarin toxicity (page 1245).<sup>16,32</sup>

In adults, the parenteral administration of 10 to 20 mg of vitamin K<sub>1</sub> abolishes coagulation abnormalities within 24 hours if they are the consequence of deficiency of this vitamin. Failure of vitamin K to normalize the prothrombin time is good evidence for the presence of a complicating process, eg, liver disease, DIC. Repeated administration of vitamin K or larger doses may be required to reverse the effects of coumarin toxicity (page 1245), but, with this exception, there is no evidence that large doses of vitamin K are any more effective than those mentioned above. The synthetic vitamins K<sub>3</sub> (menadi-ones) may be absorbed in the absence of bile salts and in various malabsorption syndromes.<sup>8</sup> However, these congeners of vitamin K have a more transient effect than the natural forms of this vitamin,<sup>17</sup> and offer little therapeutic advantage in the usual case.

Vitamin K may produce hemolytic anemia

in patients with hereditary deficiencies of various red cell enzymes (Chapter 23).

## Hepatic Disease

Virtually every hemostatic function may be impaired in patients with severe hepatic disease.<sup>25</sup> This results from deficiencies in both the biosynthetic and clearance functions of the liver. The pathophysiology of some of these abnormalities is discussed elsewhere, namely, thrombocytopenia (page 1098), fibrinogenolysis (page 1224), and the effects of products of fibrinogen catabolism on platelets and on the coagulation mechanism (page 438).

### Pathophysiology

In liver disease, *deficiencies* of prothrombin and factors VII, IX, and X result mainly from synthetic incompetence of the hepatic cells. In addition, in some cases there may also be vitamin K deficiency as the result of poor diet, or mild malabsorption caused by insufficient production of bile salts or exocrine pancreatic insufficiency.<sup>8</sup> Fibrinogen and factor V also are synthesized by the liver, but neither factor is vitamin K-dependent; both may be deficient in severe liver disease. Mild deficiencies of factors XI<sup>24</sup> and XIII<sup>36</sup> also have been reported.

In addition, it is possible that hypervolemia or accelerated catabolism contribute to the deficiencies of coagulation factors in some patients with severe liver disease.<sup>281</sup> The loss of fibrinogen into ascitic fluid may be significant in some patients.

*Endogenous plasminogen activators* normally are removed from the circulation by the liver (page 435). Consequently, in patients with severe liver disease these activators may circulate for an abnormally long time, and may produce a *state of chronic or intermittent fibrinogenolysis*. This may be an important contributory factor in the pathogenesis of hypofibrinogenemia (page 1224). In such patients, surgical procedures or trauma, certain drugs, or electroshock may induce acute fibrinogenolysis.<sup>11</sup>

There is some evidence that the cirrhotic

liver produces an *abnormal plasminogen activator*,<sup>9</sup> and that the levels of circulating antiplasmins may be subnormal in patients with cirrhosis as a consequence of deficient hepatic synthesis.<sup>31</sup> These processes are of uncertain importance in the pathophysiology of fibrinogenolysis in liver disease.<sup>11,22</sup>

The most significant consequence of fibrinogenolysis in liver disease is the *production of large quantities of fibrinogen degradation products (FDP)*, which may persist in the circulation for abnormally long periods because of deficient hepatic clearance. The effects of such FDP presumably underlie the abnormalities of platelet function that have been reported in some patients with hepatic disease.<sup>23</sup> Azotemia is common in terminal liver disease; this abnormality also impairs platelet function (Chapter 35).

Because it is associated with deficient hepatic clearance of activating substances and of products of coagulation, severe liver disease theoretically would predispose to the development of DIC. Fibrinogen catabolism often is accelerated in cirrhosis,<sup>281</sup> and may be normalized in some patients following heparin administration. However, heparin administration seldom decreases the duration or severity of bleeding in patients with severe liver disease.<sup>115</sup> Furthermore, fibrinogen catabolism may be accelerated in patients with extrahepatic obstruction of the portal vein,<sup>281</sup> in whom abnormal bleeding does not occur. Although the question has not been settled, present evidence would suggest that DIC is only rarely responsible for bleeding in patients with severe liver disease in the absence of other etiologic factors, eg, sepsis, shock, cancer.<sup>2</sup> The difficulty of distinguishing between DIC and the effects of severe liver disease by laboratory procedures is discussed on page 1221 (Table 38-2).

### Clinical Picture

In view of the numerous hemostatic abnormalities that may be present, it is surprising that many patients with severe liver disease do not bleed abnormally. Gastrointestinal hemorrhage is the commonest

bleeding manifestation, but it almost always originates from a local lesion, eg, esophageal varices, peptic ulcer, gastritis. The degree to which hemostatic abnormalities contribute to such bleeding is uncertain. In one large series,<sup>6</sup> gastrointestinal bleeding was not significantly more severe or protracted in the presence of coagulation abnormalities, but, in another study, serious hemorrhage occurred only in patients with prolonged prothrombin times and significant factor IX deficiency.<sup>26</sup> Moderate generalized bleeding manifestations, such as recurrent ecchymoses and epistaxis, are not uncommon, and severe generalized bleeding may complicate surgical procedures, including biopsies and other minor procedures. Acute fibrinogenolysis<sup>14</sup> may be the major pathophysiologic factor in such patients, and is particularly hazardous and unpredictable, since it may develop despite normal screening tests of coagulation.

### Laboratory Diagnosis

The laboratory findings in liver disease vary with the cause and severity of the underlying disorder, and range from a slight prolongation of the PTT in anicteric hepatitis to the picture summarized in Table 38-2, which is seen in severe decompensated cirrhosis. In cirrhosis, coagulation abnormalities correlate with the presence of active disease and not with the presence of portal hypertension.<sup>4</sup> Although coagulation abnormalities may be minimal in inactive cirrhosis, thrombocytopenia alone is not uncommon in association with portal hypertension. Fibrinogenolysis is significantly more common in cirrhosis than in acute hepatocellular disease, eg, hepatitis. The plasma levels of factor VIII may be elevated in hepatic disease, and may be very high in the presence of acute hepatocellular necrosis.<sup>9</sup> Coagulation abnormalities are seldom marked in biliary cirrhosis, and hemostatic abnormalities of any sort are rare in metastatic liver disease.<sup>4</sup> The presence of a qualitatively abnormal fibrinogen (acquired dysfibrinogenemia) (page 1174) has been reported in patients with hepatomas.<sup>33,35</sup>

**Table 38-2. Laboratory Findings in Acquired Coagulation Disorders**

	Hemorrhagic Disease of Newborn	Severe Liver Disease	Intravascular Coagulation	Fibrino- genolysis	Antibodies to Factor VIII	Inhibitors of SLE Type
<b>Screening tests</b>						
Platelet count (1)	N	vD	D	uN	N	V (1)
Bleeding time	N	vl	vl	vl	N	V (1)
Prothrombin time (3)	ml	ul	I	vl	N	ul
PTT (3)	ml	ul	V	vl	I	I
Thrombin time (3)	vl	ul	I	I	N	N
Coagulation time	ul	V	V	V	I	I
Erythrocyte morphology (1)	Macrocytes (3)	Target cells & macrocytes	Schistocytes & micro- spherocytes	uN	uN	uN (1)
<b>Specific assays</b>						
Fibrinogen	N	vD	D	vD	N	N
Prothrombin (3)	D	uG	V	uN	N	uD (2)
Factor V	N	uD	uD	vD	N	N (2)
Factor VII (3)	D	uD	uN	uN	N	N (2)
Factor VIII	N	ul	vD (2)	vD	mD	N (2)
Factor IX (3)	D	uD	V (2)	uN	N	N (2)
Factor X (3)	D	uD	vD	uN	N	N (2)
Factor XI (3)	vD	vD	uN (2)	uN	N	N (2)
Factor XIII (4)	uN	vD	uD	vD	N	N
<b>Tests for fibrinolysis &amp; FDP</b>						
Whole clot lysis (4)	uN	vl	uN	I	uN	N
Euglobulin clot lysis (4) (5)	uN	vl	uN	I	uN	N
FDP	uN	ul	ml	ml	N	N
Paracoagulation (plasma) (1)	uN	vA	uA	uN	uN (1)	uN (1)
Paracoagulation (serum)	uN	vA	uA	uA	N	N
Cryofibrinogen	N	vl	vl	uN	N	N
Plasminogen (3)	vD (3)	vD	D	D	N	N
Antiplasmins	uN	vD	V	vD	N	N
Plasmin	N	vl	uN	ul	N	N
<b>Miscellaneous tests</b>						
Antithrombin III	N	V	vD	uN	N	N
Platelet factor 4 in plasma	N	?	vl	uN	N	N

KEY: N = normal A = abnormal I = increased D = decreased V = variable, m = markedly u = usually v = variably

(1) May reveal effects of acute bleeding, or abnormality characteristic of underlying disease

(2) One stage assays may yield aberrant results

(3) Results may differ from adult norms in normal neonates

(4) Hypofibrinogenemia may alter results

(5) Plasminogen depletion may alter results

## Treatment

Vitamin K produces some improvement in the coagulation abnormalities in approximately 30% of patients with liver disease.<sup>26</sup> Large doses of vitamin K may prolong the prothrombin time in some subjects<sup>8</sup>; the explanation of this paradoxical effect is obscure.

Replacement therapy is often disappointing in liver disease.<sup>26</sup> Factors that may explain this include the very short *in vivo* half-life

of factor VII, hypervolemia, and the fact that the *in vivo* recovery of transfused factor IX is significantly lower than that of other factors, even in the absence of liver disease (Table 37-5, page 1184). In general, replacement therapy is indicated only in the presence of serious bleeding, or prior to surgical procedures. Fresh plasma is preferable, but the effects of even maximum doses (15–20 ml/kg of body weight) are often transient.<sup>3</sup>

*Antifibrinolytic agents* may be indicated in patients with fibrinogenolysis. The clinical effects of these drugs are hard to assess<sup>13</sup> in chronic liver disease, but good results have been obtained in the treatment and prevention of acute postsurgical bleeding, particularly that associated with portacaval shunts.<sup>11</sup> Thromboembolic complications have been reported following EACA administration in some patients

## Pathologic Inhibitors of Coagulation

The pathologic inhibitors of coagulation or circulating anticoagulants may be defined as "abnormal endogenous components of blood that inhibit the coagulation of normal blood."<sup>79</sup> They may act at virtually any stage in the process of coagulation. It is now clear that some inhibitors are antibodies, but with this exception the nature and mode of action of these substances are poorly understood.<sup>79</sup>

### Antibodies to Specific Factors

Antibodies to coagulation factors usually act as specific inhibitors; ie, they inactivate a single factor. They produce a clinical and laboratory picture that resembles a hereditary coagulation disorder in many respects. Such antibodies have provided an important new research tool that is of particular value in the study of the qualitatively abnormal forms of the various coagulation factors (page 1160).<sup>73</sup>

### Antibodies to Factor VIII

Antibodies to factor VIII are the most common of the specific inhibitors. They have been demonstrated in from 5<sup>42</sup> to 21%<sup>79,86</sup> of patients with hemophilia A, most commonly in those who are severely affected. Rarely, they complicate mild forms of the disorder.<sup>40,52</sup> Antibodies to factor VIII also arise spontaneously in association with vari-

ous chronic inflammatory diseases such as rheumatoid arthritis, systemic lupus erythematosus, and ulcerative colitis<sup>93</sup>; and in elderly persons in the absence of apparent underlying disease.<sup>96</sup> Less commonly antibodies are found in association with drug reactions,<sup>67</sup> pregnancy and the puerperium,<sup>79,93</sup> and various other disorders.<sup>63</sup>

### Pathophysiology

In patients with hemophilia A, the development of antibodies presumably is related in some way to replacement therapy with factor VIII, but the immunologic mechanism remains unclear.<sup>41</sup> It has been clearly established, however, that, once an antibody has developed in a patient with hemophilia A, administered factor VIII acts as an antigen.<sup>92</sup> The "immunizing events" leading to the development of antibodies of the spontaneous type remain obscure.

Most antibodies to factor VIII are immunoglobulins of the IgG type.<sup>57,93</sup> In one patient with multiple myeloma,<sup>63</sup> an IgA protein acted as an inhibitor of factor VIII, and one inhibitor of the spontaneous type was an IgM immunoglobulin.<sup>51</sup> In some patients, inhibitory activity is present in both the IgG and IgM proteins.<sup>73</sup> In many, the light chains of the antibody appear to be entirely of one type, ie, kappa<sup>93</sup> or lambda.<sup>59</sup> Such "monotypic" antibodies could not be demonstrated by others.<sup>85,96</sup>

These antibodies inactivate factor VIII by means of a time and temperature dependent reaction, the kinetics and stoichiometry of which are quite variable.<sup>43,73,85,93</sup> There is some evidence that antibodies arising in patients with hemophilia A have different kinetic properties than those that form spontaneously.<sup>67</sup> In most subjects, the inactivation of factor VIII is irreversible, but, with antibodies of low affinity, the factor VIII-antibody complex may dissociate in vitro. This phenomenon may produce aberrations in the assay system, and may explain certain puzzling laboratory features in "atypical" cases.<sup>41,45</sup>

## Clinical and Laboratory Features

The *bleeding manifestations* resulting from antibodies to factor VIII are virtually identical to those seen in hemophilia A, and may be severe (page 1164). It is probable that such inhibitors explain some reports of hemophilia in the female. Massive hemorrhage following trivial trauma, spreading hematomas in soft tissues, spontaneous and intractable epistaxis, and even hemarthrosis may occur.<sup>95</sup> When antibodies arise in patients with mild hemophilia A, bleeding typical of severe deficiency may develop.<sup>40</sup> More significantly, the patient may become virtually refractory to replacement therapy. This may have serious and even fatal consequences.<sup>87</sup> In one patient who developed antibodies to factor VIII during pregnancy, the inhibitor crossed the placenta.<sup>60</sup> Rarely, antibodies to factor VIII are associated with thrombocytopenia.<sup>102</sup>

The *laboratory findings* resemble those observed in persons with hemophilia A (Table 38-2). Factor VIII levels usually are nil in severely affected patients but range up to 10% or even higher. Specific tests for antibodies involve the demonstration of progressive and time-dependent inactivation of factor VIII in vitro by the plasma or serum of the patient. Simple mixing techniques based on the PTT, the recalcification time, and the coagulation time have been described<sup>79</sup> (page 1062), and several methods for quantifying the levels of antibody have been devised.<sup>43,87,98,100</sup> An in vivo screening test for antibodies to factor VIII also has been described.<sup>97</sup>

Antibodies to factor VIII do not fix complement.<sup>88</sup> Presumably because the factor VIII-antibody complex is soluble under most conditions, precipitation and immunodiffusion methods usually have yielded negative results. Positive serologic results were obtained with hemagglutination methods in one case.<sup>89</sup>

## Treatment

Huge amounts of factor VIII concentrates (20,000 to 200,000 units) produced apparent

remission of bleeding in some patients,<sup>45,55</sup> but many others have failed to respond. In one series,<sup>85</sup> the effectiveness of factor VIII administration appeared to be determined by the affinity of the antibody for factor VIII. Therapy with factor VIII was seldom successful in patients with high-affinity antibodies and infinite coagulation times.<sup>95</sup> It has been suggested that in such patients the use of factor VIII concentrates of bovine or porcine origin may offer a significant therapeutic advantage, since such heterologous factor VIII is less reactive with most antibodies than is human factor VIII.<sup>98</sup> Continuous slow infusion of factor VIII may be more effective than the usual intermittent regimens, owing to the time-dependent nature of the antibody-factor VIII interaction.<sup>95</sup>

In most patients with hemophilia A who have a factor VIII antibody, infused factor VIII provokes a rapid rise in the titer of antibody.<sup>55,87</sup> Consequently, replacement therapy with factor VIII should be avoided in such patients if at all possible. There is no direct evidence that infusion of factor VIII has a similar effect in patients with inhibitors of the spontaneous type.

With a few exceptions,<sup>94</sup> corticosteroids have proved therapeutically ineffective.<sup>41,79</sup> The adjunctive value of exchange transfusions and immunosuppressive drugs has not been thoroughly evaluated.<sup>92,95</sup> The results of combined therapy with immunosuppressive drugs and massive doses of factor VIII have been encouraging.<sup>55,68</sup> Spontaneous remissions may occur in a significant number of patients, particularly when the antibody developed following penicillin therapy<sup>67</sup> or during pregnancy.<sup>79</sup>

## Miscellaneous Specific Inhibitors

Specific inhibitors that are directed against several other coagulation factors have been well documented. The available evidence suggests that these inhibitors also are antibodies.<sup>56</sup> Inhibitors of factor IX are very rare,<sup>72,82,84</sup> and have been demonstrated only in patients with hemophilia B. In contrast to

antibodies to factor VIII, they act instantaneously. An immunoabsorbent compound has been prepared from one such antibody to factor IX.<sup>82</sup> In one kindred, an inhibitor of factor IX appeared to be the result of an inherited trait.<sup>61</sup> Inhibitors of factor V have developed in several patients.<sup>58,59</sup> In contrast to most other specific inhibitors, they seldom produce serious hemorrhage<sup>56</sup> and disappear spontaneously in most cases. In several patients, the development of inhibitors of factor V was associated with the administration of streptomycin.<sup>56</sup> A precipitating antibody to fibrinogen has been demonstrated in patients with hereditary afibrinogenemia following transfusions.<sup>47,54</sup> Specific inhibitors of factors XI and XII<sup>30,51</sup> and of the activated form of factor XI<sup>49</sup> have been described in association with systemic lupus erythematosus.

Specific inhibitors of factor XIII have been described following transfusions in patients with hereditary deficiencies of this proenzyme<sup>64,77</sup> and in previously normal persons.<sup>66,78,80</sup> Many of the latter had received isoniazid,<sup>74,75</sup> and it was suggested that this drug may alter factor XIII in such a manner that it becomes anigenic.<sup>75</sup> These inhibitors may impair the activation of factor XIII, or may be directed against the cross-linking sites of fibrin.<sup>90</sup>

### Inhibitors in Systemic Lupus Erythematosus

A unique type of coagulation inhibitor is commonly associated with systemic lupus erythematosus. These "lupus anticoagulants" have been detected in from 10 to 15% of patients in several large series.<sup>56</sup> The inhibitor alone seldom produces symptoms and, in most patients in whom bleeding was observed, it appeared to be the result of coexistent thrombocytopenia, uremia, or prothrombin deficiency.<sup>56</sup>

Despite intensive study, the lupus anticoagulants remain poorly understood. They are immunoglobulins of either the IgG or IgM class, but there is little evidence that they are antibodies. They act instantaneously rather than in a time-dependent manner, and, when

a specific reaction is measured, they appear to affect the rate rather than the yield. In some cases, a plasma cofactor apparently is required for their inhibitory action.<sup>103</sup> As a consequence of the foregoing, these inhibitors are very difficult to study, and their mode of action has been variously attributed to inhibition of the interaction between factors V and X,<sup>46</sup> inhibition of the interaction between factors IX and VIII,<sup>44</sup> and antagonism of prothrombinase.<sup>103</sup> Most data favor the last view.<sup>56,83,71,86</sup> There is indirect evidence that the lupus anticoagulants may be directed against the phospholipid moiety of prothrombinase.<sup>56</sup>

The results of laboratory tests (Table 38-2) that employ undiluted blood or plasma, eg, the whole blood coagulation time, thrombin generation test,<sup>44</sup> PTT, may show marked deviation from normal. The effects of the inhibitor often are diminished or abolished by dilution. The prothrombin time usually is prolonged, an abnormality that may be magnified by dilution of the tissue thromboplastin in some cases.<sup>79</sup> The results of the thromboplastin generation test usually are normal except when the patient's plasma is used as substrate. A moderate deficiency of prothrombin has been reported in most cases,<sup>44</sup> but this additional abnormality cannot be clearly related to the presence of the inhibitor. With this exception, the results of specific assays for various coagulation factors usually are normal, although the inhibitor may produce artifactual abnormalities in one-stage assay systems.

Spontaneous remissions are uncommon,<sup>56</sup> but treatment is seldom required. Corticosteroids<sup>79</sup> and immunosuppressive drugs<sup>49</sup> diminish or abolish the coagulation abnormalities in many patients.

### Miscellaneous Inhibitors

Bleeding has been attributed to presence of antithrombins in patients with various disorders, most commonly in those with liver disease. It would now seem that, in most of these patients, such antithrombins were, in fact, FDP and were the result of underlying

intravascular coagulation or fibrinogenolysis. The physiologic antithrombins are discussed on page 431.

In multiple myeloma and other *paraproteinemias*, abnormal proteins may be absorbed by fibrinogen or fibrin. They act as antithrombins and also as inhibitors of fibrin polymerization, and result in gelatinous, structurally abnormal clots.<sup>50,59</sup> The formation of complexes between other coagulation factors and abnormal proteins has been demonstrated *in vitro*.<sup>70</sup> It is difficult to assess the clinical significance of coagulation abnormalities in the paraproteinemias. Laboratory abnormalities seldom correlate closely with the severity of bleeding, and coexistent abnormalities such as hypercoagulability and fibrinolysis,<sup>62,83</sup> thrombocytopenia, platelet dysfunction, and uremia are commonly present.

The presence of large amounts of FDP also may explain some reports of circulating "heparin-like" anticoagulants. In most cases, heparin was implicated merely because protamine reversed the coagulation abnormalities *in vitro*,<sup>79</sup> an effect that can be produced in many disorders in which large amounts of FDP are present in the circulation. It remains uncertain whether heparin in concentrations sufficient to affect coagulation ever is released from endogenous sites in man.

An anticoagulant has been isolated from leukocytes of patients with chronic myelocytic leukemia.<sup>69</sup>

## Diffuse Intravascular Coagulation

The syndrome of diffuse intravascular coagulation or DIC (disseminated intravascular coagulation, defibrination syndrome, consumption coagulopathy,<sup>250</sup> intravascular coagulation-fibrinolysis [ICF] syndrome<sup>234</sup>) has been one of the most intensively studied subjects in the field of hematology during the last decade, and investigation of the more general importance of DIC as an "intermediary mechanism of disease"<sup>208</sup> has led far afield. The development of new and highly

sensitive diagnostic techniques has resulted in the recognition of DIC in a seemingly endless variety of clinical situations.

### Etiology and Incidence

DIC has been well documented in association with the disorders summarized in Table 38-3. The true incidence of the syndrome is unknown. In many of the disorders listed, DIC develops only in an occasional case. Thus, it is rare in heat stroke,<sup>115,263</sup> in "auto-immune" disorders,<sup>140</sup> and in hemolytic anemias.<sup>109</sup> The incidence of DIC in association with many disorders appears to be proportional to the energy with which the diagnosis is pursued, eg, septicemia, intrauterine fetal death.

Available evidence does not justify the conclusion that DIC is a major pathophysiologic feature in thrombotic thrombocytopenic purpura<sup>170,196</sup> (page 943), eclampsia,<sup>239</sup> the hemolytic uremic syndrome,<sup>170,283a</sup> various forms of chronic renal disease,<sup>236</sup> and severe liver disease.<sup>115</sup> That many hold the opposite view should be noted, however, and the pathophysiologic importance of DIC in these disorders remains unsettled. Further details are given elsewhere.<sup>115,143,153,167,208,212,236,250</sup>

### Pathophysiology

The pathophysiology of DIC is complicated and poorly understood.<sup>218</sup> Acute DIC usually is a medical emergency; complete studies are difficult to obtain, and, even in cases that can be carefully evaluated, the physician is confronted with the end result of a complex sequence of interrelated events that can seldom be reconstructed. As a result, present concepts in the main are based on *in vitro* experiments and studies in lower animals. The relevance of such data to human disease has yet to be clearly established.

The mechanisms by which DIC is initiated are numerous,<sup>223</sup> but all have in common the capacity, in terms of either the magnitude or the duration of the activating stimulus, to exceed or "overwhelm" normal compensa-

**Table 38-3. Causes of Diffuse Intravascular Coagulation**

- ✓ I **Obstetric Complications** (abruptio placentae, septic abortion and chorioamnionitis, amniotic fluid embolism, intrauterine fetal death, miscellaneous [degenerating hydatidiform moles and leiomyomas, intra amniotic saline injections,<sup>218</sup> abdominal pregnancy,<sup>211</sup> tetracycline induced hepatorenal failure,<sup>219</sup> fetomaternal blood passage, & severe eclampsia])
- II **Infections**
  - A **VIRAL** (herpes rubella smallpox<sup>215</sup> cytomegalic inclusion disease<sup>174</sup> various epidemic hemorrhagic fevers<sup>211</sup> others)
  - B **RICKETTSIAL** (Rocky Mountain spotted fever<sup>220</sup> others)
  - C **BACTERIAL** (meningococcemia septicemia particularly that due to gram-negative organisms, many others)
  - D **MYCOTIC** (histoplasmosis aspergillosis<sup>146</sup>)
  - E **PROTOZOAL** (malaria<sup>142</sup> trypanosomiasis<sup>122</sup>)
- ✓ III **Neoplasms**
  - A **CARCINOMAS** (prostate pancreas breast lung ovary,<sup>222</sup> many others)
  - B **MISCELLANEOUS** (metastatic carcinoid<sup>145</sup> rhabdomyosarcoma<sup>216</sup> neuroblastoma,<sup>217</sup> others<sup>228</sup>)
- IV **Disorders of the Hematopoietic System**
  - A **ACUTE LEUKEMIA** (promyelocytic,<sup>193</sup> other types<sup>127</sup>)
  - B **INTRAVASCULAR HEMOLYSIS** (transfusion of incompatible blood<sup>200</sup> 223 paroxysmal nocturnal hemoglobinuria other disorders rarely<sup>115</sup>)
- V **Vascular Disorders**
  - A **MALFORMATIONS** (giant hemangiomas [Kasabach-Merritt syndrome<sup>125</sup> 143] aneurysms of the aorta,<sup>141</sup> and other large vessels cyanotic congenital cardiac lesions)
  - B **COLLAGEN VASCULAR DISORDERS** (acute vasculitis<sup>179</sup> polyarteritis<sup>141</sup> systemic lupus erythematosus rarely<sup>215</sup>)
  - C **HYPOXIA AND HYPOPERFUSION** (congestive failure with pulmonary emboli rarely,<sup>211</sup> cardiac arrest,<sup>142</sup> various forms of shock<sup>116</sup> hypothermia<sup>119</sup>)
- VI **Coincident to Massive Tissue Injury** (large traumatic injuries and burns,<sup>156</sup> 214 278 extensive surgery<sup>116</sup> 219 extracorporeal circulation fat embolism<sup>111</sup>)
- VII **Miscellaneous** (amyloidosis<sup>110</sup> snakebite<sup>150</sup> anaphylaxis<sup>128</sup> drug reactions<sup>214</sup> heat stroke<sup>263</sup> purpura fulminans<sup>174</sup> severe respiratory distress syndrome<sup>203</sup> diabetic acidosis,<sup>190</sup> Cushing's syndrome,<sup>278</sup> acute pancreatitis<sup>182a</sup>)

\*May produce a similar syndrome in the fetus as a result of maternal to fetal blood passage.<sup>17</sup> 191

tory processes (Fig. 38-2). Thrombin is persistently or recurrently elaborated within the circulation, and the process of DIC, like normal coagulation, becomes "autocatalytic." Fibrinogen, various other coagulation factors, and platelets are utilized, and fibrin formation begins. The fibrinolytic enzyme system is activated, and large amounts of fibrin degradation products (FDP) are produced. These further impair hemostatic function. Bleeding, shock, and vascular occlusion commonly supervene, and produce profound alterations in the function of virtually every organ system. Normal compensatory processes may become impaired so as to create a self-perpetuating "vicious cycle." The ultimate outcome is determined by a dynamic interplay between the various pathologic processes and compensatory mechanisms, ie, fibrin deposition vs fibrinolysis; depletion vs reple-

tion of coagulation factors and platelets; production vs clearance of fibrin, FDP, and other products of coagulation.

### **Mechanisms by Which DIC is Initiated**

The mechanisms that activate or "trigger" DIC act upon pathways that are involved in normal hemostasis, namely, the processes of platelet adhesion and aggregation, and the contact and tissue-activated pathways of coagulation. In most forms of DIC the initiating factors are multiple and interrelated. For example, in meningococcemia, endothelial injury may lead to the release of thromboplastins and collagen exposure which initiates platelet adhesion, aggregation, and contact activation; and endotoxemia also produces platelet aggregation and contact activation.



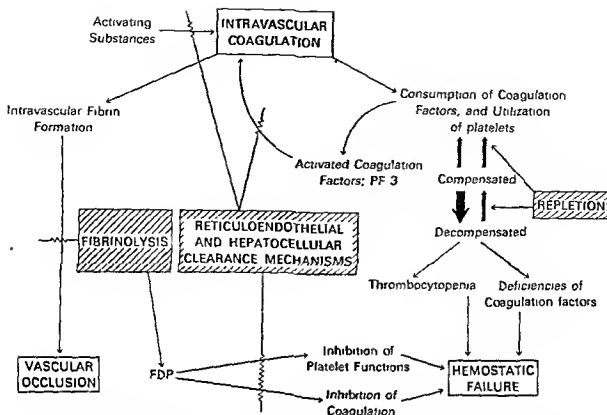


Fig 38-2. The pathophysiology of diffuse intravascular coagulation. The major compensatory processes are illustrated in hatched blocks.

**THROMBOPLASTIC SUBSTANCES.** The entry of thromboplastic substances into the general circulation from endogenous sites ("autoinfusion") presumably is a contributory factor in most forms of DIC, and is of major pathogenetic importance in cases associated with abruptio placentae, intrauterine fetal death, amniotic fluid embolism,<sup>256</sup> and various neoplasms. In abruptio placentae,<sup>273</sup> decidual fragments, serum containing activated coagulation factors, and other material from the placental site enter the intervillous "maternal lake" and hence the venous circulation. This process is initiated by rupture of the basal decidual plate, and has been termed "auto-extraction."<sup>256</sup> The deposition of fibrin in the retroplacental clot is of minor importance, even though such clots may be massive.<sup>239</sup>

In amniotic fluid embolism, relatively weak thromboplastins together with large amounts of particulate matter enter the circulation suddenly. If the patient survives this circu-

latory catastrophe, DIC develops. In intra-uterine fetal death, thromboplastic substances from the dead fetus are slowly but continuously absorbed, producing a picture of chronic but progressive DIC<sup>239</sup>; rarely, acute DIC with marked fibrinogenolysis may be associated.<sup>240</sup> In neoplasms, breakdown products from the tumor and tumor micro-emboli are thought to enter the circulation and act as thromboplastins. Some neoplasms secrete thrombin-like enzymes,<sup>190a</sup> or fatty acids which alone, or when combined with albumin,<sup>154</sup> act as thromboplastins.<sup>233</sup>

In DIC associated with massive trauma,<sup>209</sup> major surgical procedures, or large burns, thromboplastic substances from damaged tissues are presumably the major initiating factor. In such cases, antecedent abnormalities ("hypercoagulability," azotemia) and complications (shock, intravascular hemolysis, massive transfusions of stored blood (page 1102), septicemia, hypoxia) are important con-

tributory factors.<sup>114,136</sup> Various circulatory-assist devices that are employed during surgical procedures may produce hemolysis, activation of factor XII, platelet damage, and denaturation or utilization of fibrinogen and other coagulation factors.<sup>278</sup>

**INFECTIONS.** The frequency with which DIC accompanies meningococcemia and septicemia due to other bacteria that possess potent endotoxins has led to intensive study of the effects of endotoxin on the hemostatic apparatus. Purified *endotoxin* produces several effects that may lead to DIC, namely, activation of factor XII,<sup>204</sup> platelet aggregation,<sup>245</sup> inhibition of fibrinolysis, leukocyte aggregation, direct endothelial injury,<sup>207</sup> and impairment of compensatory clearance functions.<sup>123</sup> The coagulation abnormalities and the histopathologic appearance of the lesions produced by endotoxin in the generalized Sanarelli-Shwartzman phenomenon in animals resemble those found in human DIC.<sup>287</sup> Studies of this animal model have been intensive,<sup>123,205,223,232,249,251</sup> and have revealed the following of particular pertinence to DIC in man: (1) Shwartzman-like phenomena may be produced by agents other than endotoxin, such as factor Xa,<sup>251</sup> substances that produce contact activation and platelet aggregation,<sup>214</sup> and purified platelet factor  $3^{249}$ ; (2) the infusion of FDP, tissue thromboplastin, red cell hemolysates, and inhibitors of fibrinolysis produces changes that simulate the effects of reticuloendothelial blockade; (3) heparin, coumarin anticoagulants, granulocytopenia,<sup>197</sup> thrombocytopenia,<sup>202</sup> activators of endogenous fibrinolysis, and inhibitors of factor XII activation inhibit the Shwartzman phenomenon; (4) continuous slow infusion of endotoxin produces DIC in the absence of either a "priming dose" or reticuloendothelial blockade<sup>5</sup>; and (5) the Shwartzman phenomenon can be produced in pregnant animals with only one dose of endotoxin. The pertinence of these data to human disease nevertheless remains uncertain.<sup>115</sup> The Shwartzman phenomenon and the effects of endotoxin in general are highly species specific, the pathophysiologic role of endotoxi-

nemia in human sepsis is by no means clear,<sup>206</sup> and DIC may complicate acute infections due to bacteria lacking potent endotoxins, eg, pneumococci.<sup>126,286</sup> In meningococcemia and other forms of endotoxemia,<sup>207</sup> direct vascular injury results in the release of thromboplastins, and even endothelial cells,<sup>129</sup> into the general circulation, and may be the major factor in the initiation of DIC. A similar phenomenon may be involved in rickettsial and viral infections. In malaria,<sup>142</sup> massive intravascular hemolysis may occur. In most severe infections, the complicating influence of "septic" shock may be considerable, as will be discussed below.

**SHOCK AND HYPOPERFUSION.** The relationship between various forms of shock and DIC has not been clearly defined. It has been suggested that DIC is involved in all forms of shock, and that it is the central feature in irreversible or refractory forms.<sup>167,168</sup> However, the evidence that uncomplicated hemorrhagic shock initiates DIC is unconvincing.<sup>114,234</sup> In septic shock, the interrelationships between septicemia, shock, and endotoxemia are exceedingly complex, and studies in lower animals must be interpreted with caution because of marked and unexplained differences in the responses of various species.<sup>138</sup> In septicemia, both thrombocytopenia and "septic shock" may occur without DIC. An overriding problem in interpreting all of these data is the difficulty in distinguishing between causes and effects.

Although the importance of shock as a "trigger" factor in DIC is unclear, shock may nevertheless play an important pathophysiologic role. Hypoperfusion, even of normal vessels, acidosis,<sup>135</sup> and hypoxemia produce "hypercoagulability" and favor platelet aggregation.<sup>115</sup> Furthermore, splanchnic hypoperfusion produces a marked impairment of reticuloendothelial and hepatic clearance functions, and is present in virtually all forms of shock. Thus, shock may favor the development of DIC from activating stimuli that ordinarily would not exceed the capacity of compensatory processes, and may perpetuate

DIC, after a transient activating stimulus has been dissipated. Shock also may impair hepatic synthesis of coagulation factors, and thus contribute to the coagulation defect in DIC.

DIC in association with giant hemangiomas (Kasabach-Merritt syndrome),<sup>165,175</sup> or with aneurysms of the aorta<sup>181</sup> or other large vessels,<sup>125</sup> has been attributed to *hypoperfusion of local vascular beds*. The pathophysiologic role of hypoxemia in DIC associated with cyanotic congenital heart disease is obscure.<sup>124</sup>

**MISCELLANEOUS ACTIVATING STIMULI.** There is good experimental evidence that various processes in addition to those described above may initiate DIC, and multiple "trigger" factors usually complicate the clinical situation. Thus, factor XII is activated by saturated fatty acids in vitro, but the importance of such lipids in the pathogenesis of fat embolism remains unclear.<sup>131</sup> Antigen-antibody complexes produce platelet aggregation and also activate factor XII in vitro, and it has been suggested that such complexes, or cytotoxic antigen-antibody reactions, play an important role in DIC associated with acute anaphylactic reactions,<sup>219</sup> purpura fulminans (page 1219),<sup>174</sup> and transfusion of incompatible blood.<sup>247</sup> When incompatible blood has been given, the release of ADP from hemolysed red cells also may be involved.<sup>236</sup> The infusion of trypsin and other proteolytic enzymes may induce DIC in animals, and the venoms of various snakes contain similar enzymes. Crude venoms also contain substances that act as thromboplastins, and produce intravascular red cell hemolysis and massive vascular damage.<sup>250</sup>

The pathophysiology of DIC in association with acute leukemia is unclear. This syndrome has been described in all of the morphologic forms,<sup>118,137,147,193</sup> but is most common in the promyelocytic variety.<sup>272</sup> The granules of various "blast" forms contain thromboplastic substances,<sup>160,161</sup> the promyelocyte containing particularly high concentrations.<sup>161</sup> Lysosomal fractions of myeloblasts and promyelocytes exhibit fibrinolytic ac-

tivity in vitro, whereas those of lymphoblasts are procoagulant.<sup>137</sup> In one patient, lysozymes were isolated from the urine, and it was suggested that these originated in leukemic cells and were the cause of DIC.<sup>193</sup>

### *Consumption of Coagulation Factors*

DIC constitutes a model of accelerated turnover of various coagulation factors, whose levels at any time are determined by the size of the plasma pool and the differences between the rates at which they are being destroyed and replenished. Quantitative studies have demonstrated accelerated turnover rates for platelets, fibrinogen, and prothrombin.<sup>170,185,234</sup> There are no quantitative data concerning other coagulation factors. Numerous factors complicate a simple kinetic approach. For example, circulating FDP may induce the release of large amounts of fibrinogen into hepatic lymph,<sup>121</sup> and also increase the rate of fibrinogen synthesis as much as four-fold.<sup>185</sup> Other complexities include impaired hepatic synthesis of coagulation factors, and the phenomenon of post-depletion "rebound."

Generalizations based on the consumption of coagulation factors during in vitro coagulation (page 430) (Table 10-2, page 411) are not always consistent with laboratory findings in DIC.<sup>229</sup> Thus, factors that normally are not consumed may be deficient in DIC; eg, factors VII, IX, X. Prothrombin, which is completely consumed in vitro, often is normal in DIC. Significant hypoprothrombinemia must reflect either massive and protracted activation of coagulation or complicating factors, since, in animals, severe depletion of platelets, fibrinogen, and factors V and VIII results from activation of only 10% of plasma prothrombin.<sup>243</sup>

Substances other than coagulation factors may be depleted as a result of DIC. Those of homeostatic importance include antithrombin III<sup>282</sup> and plasminogen. The latter has great avidity for fibrin, and may coprecipitate in fibrin thrombi.<sup>246</sup>

### Utilization of Platelets

In DIC, the platelet count often is depressed out of proportion to coagulation abnormalities. This presumably reflects the limited production capacity of the megakaryocyte. Thrombocytopenia may result from processes other than the utilization of platelets in thrombotic lesions.<sup>236</sup> These processes include adhesion to denuded or damaged endothelial surfaces and intravascular aggregation with subsequent sequestration, which may be caused by endotoxin,<sup>123</sup> antigen-antibody complexes, thrombin, particulate matter,<sup>283</sup> and possibly fibrin-FDP complexes.<sup>120</sup>

All of the factors described above initiate the platelet release reaction<sup>155</sup> (page 394). The resulting platelet factor 3 activity may further accelerate the process of DIC,<sup>249</sup> and may contribute to impairment of clearance functions. Epinephrine and 5-hydroxytryptamine (serotonin) are released from the platelets and may reach extremely high concentrations in hypoperfused vascular beds. This process may produce sustained constriction of the afferent renal arteriole and may predispose to cortical necrosis. 5-Hydroxytryptamine also may produce pulmonary<sup>271</sup> and cerebral hypoperfusion.<sup>114</sup> The hypotensive effects of bradykinin and kallikrein,<sup>205</sup> which are activated by factor XIIa and by plasmin, and obstruction of the pulmonary vasculature produced by fibrin,<sup>230</sup> also may be significant.<sup>205</sup>

### Fibrin Formation

The formation of fibrin, in the form of small strands and "microclots,"<sup>253</sup> is the immediate result of DIC; the ultimate consequence of this process is determined by a balance between the rate of fibrin formation and the rate at which it is cleared from the circulation or lysed by the fibrinolytic enzyme system. Vascular occlusion, if it develops, presumably is the result of "embolic" thrombosis.<sup>211</sup> The localization of fibrin thrombi in animals with experimentally induced DIC can be varied in a remarkably

specific manner by pretreatment with various agents.<sup>210</sup> For example, sympathomimetic amines and angiotensin<sup>283a</sup> promote renal cortical involvement; corticosteroids favor fibrin deposition in the adrenal gland. Ischemic necrosis of certain tissues, particularly the renal cortex,<sup>218</sup> may result from transitory or low-grade DIC. It has been suggested that, in purpura fulminans, the initiating infection, or antigen-antibody complexes formed as a result thereof, serves to "condition" the skin in such a manner that it becomes susceptible to ischemic necrosis during subsequent intravascular coagulation.<sup>174</sup> This phenomenon has been compared to the local Schwartzman phenomenon and the Arthus phenomenon.<sup>175</sup>

Erythrocytes are mechanically injured during passage through fibrin networks in the microcirculation. Such *microangiopathic hemolysis* leads to the production of schistocytes and microspherocytes (Chapter 28).

### Fibrinolysis

Fibrinolysis is present in virtually every patient with DIC,<sup>219</sup> but plays a homeostatic rather than a pathologic role. It may be activated by several mechanisms. The major endogenous source of plasminogen activators is in the vascular endothelium of the microcirculation and, in DIC, such activators are apparently released as a result of endothelial injury or hypoxia. Most of the thromboplastic substances that initiate DIC also contain plasminogen activators,<sup>235</sup> eg, amniotic fluid, tumor tissues, extracts of leukemic cells.<sup>137</sup> There is good experimental evidence that the release of plasminogen activators from platelets and leukocytes also may be significant.<sup>138</sup> Finally, factor XIIa and thrombin activate plasminogen directly.<sup>232</sup>

*Fibrinolysis* must be clearly distinguished from the process of *fibrinogenolysis* in which fibrinogen and other coagulation factors are proteolytically destroyed in the circulation. Fibrinogenolysis is uncommon in DIC,<sup>115,179,219</sup> and when present is usually transitory and is overshadowed by marked

fibrinolysis. Disorders in which fibrinogenolysis presumably arises in the absence of DIC are discussed below.

### *Fibrin Degradation Products*

The step-wise process by which fibrin is proteolytically degraded and the biologic effects of the various products of the process (FDP) were discussed in Chapter 10 (Fig. 10-8, page 437).<sup>201</sup> These protein fragments inhibit several steps in coagulation,<sup>151,152,261</sup> produce a structurally defective fibrin polymer,<sup>107,119</sup> impair platelet functions<sup>269</sup> (page 438), and may constrict the pulmonary vasculature.<sup>122a,156</sup> It has now become apparent that the presence of large amounts of FDP in the circulation is a major factor in the production of hemorrhage in patients with DIC.

A large amount of fibrin remains in a soluble state as a consequence of the formation of complexes between fibrin monomers, various FDP, and fibrinogen (page 438). This has been regarded as a final defense against vascular occlusion.<sup>225</sup> In vitro, the complexes dissociate in the presence of alcohol<sup>133</sup> or protamine sulfate<sup>237</sup> to form gels or precipitates of various types (*paracoagulation*). There is indirect evidence that basic proteins from the granules of neutrophils<sup>172</sup> and factor 4 released from platelets<sup>199</sup> (page 398) may act as paracoagulants in vivo; ie, they may convert soluble fibrin-FDP complexes into particulate fibrin.

### *Impairment of Clearance Mechanisms*

The numerous processes that normally remove procoagulant material from the circulation (page 432) are of the utmost importance in DIC because of the presence of massive amounts of both activators and products of coagulation. There is good evidence that most of the products of intravascular coagulation (free fibrin,<sup>236</sup> prothrombinase,<sup>268</sup> platelet factor 3 activity, various types of FDP and complexes thereof<sup>156</sup>), as well as various initiators of the process (tissue

fragments, endotoxin, antigen-antibody complexes, thromboplastins, red cell stroma<sup>268</sup>) are removed from the circulation by the reticuloendothelial system,<sup>184,248</sup> the Kupfer cells of the liver being of particular importance (Fig. 38-2). In certain forms of DIC, large amounts of relatively inert particulate matter place an additional burden on the reticuloendothelial system, eg, amniotic fluid embolism. The hepatic cells are of primary importance in the clearance of activated coagulation factors (IXa,<sup>144</sup> Xa, and XIa<sup>115</sup>). It has been suggested that, in DIC, various substances saturate and produce an "autoblockade" of reticuloendothelial and hepatic clearance functions in a manner comparable to that produced experimentally in the Schwartzman reaction.<sup>194,223</sup> This may be an important pathophysiologic factor, particularly in perpetuating DIC following a transient activating stimulus. Other factors may indirectly contribute to "autoblockade" of clearance functions, including shock and endotoxemia, both of which produce marked hepatic hypoperfusion.<sup>123,138</sup>

### *Chronic or "Compensated" DIC*

Certain forms of DIC result from a weak or intermittent activating stimulus. In such patients, a balance is reached between destruction and production of coagulation factors and platelets (Fig. 38-2). The pathophysiology of such chronic, subacute, or "compensated"<sup>234</sup> DIC is fundamentally the same as that in the acute case. The distinction is nevertheless valuable, since the clinical picture and laboratory findings in the chronic form are more variable than in the acute one and may be diagnostically confusing.

Chronic DIC has been described in most cases of intrauterine fetal death and giant hemangioma (Kasabach-Merritt syndrome),<sup>169</sup> and in many cases of disseminated cancer. Other etiologic factors include various forms of vasculitis,<sup>181</sup> acute leukemia,<sup>194</sup> aneurysms,<sup>181</sup> hemangiomatous transformation of the spleen,<sup>260</sup> degenerating leiomyomas and hydatidiform moles, amyloidosis,<sup>130</sup> and metastatic carcinoid.<sup>145</sup>

Chronic DIC has many clinical and laboratory features that resemble an intermediate stage between the "hypercoagulable state" (page 1237) and acute DIC, an observation of more than theoretic interest. In cancer, a virtually continuous spectrum of clinical and laboratory features has been described; these range from recurrent thrombosis with high levels of platelets and coagulation factors to acute DIC with severe hemorrhage. There is incontrovertible clinical and experimental evidence that pregnancy, the best studied form of "hypercoagulability," is associated with a marked propensity for the development of DIC.<sup>223,286</sup> Indeed, it has been suggested that even normal pregnancy is a form of "low-grade" DIC,<sup>184</sup> and tissue thromboplastin can be recovered from the serum of normal postpartum women.<sup>277</sup> It is thus possible that the hypercoagulable state represents merely very slow intravascular coagulation, as discussed on page 1237.

#### *DIC in Neonates and Infants*

Several disorders unique to the neonate and infant may be associated with DIC.<sup>191,284</sup> The transplacental passage of thromboplastin or other activating substances was the apparent cause of DIC in neonates born of mothers affected with DIC due to abruptio placentae,<sup>149</sup> toxemia,<sup>195</sup> or septicemia. The development of DIC in a neonate following the intrauterine death of a twin fetus was attributed to "fetofetal" passage of thromboplastins.<sup>221</sup> Generalized virus infections (herpes simplex, cytomegalic inclusion disease, rubella) are common causes of DIC in infants.<sup>171</sup> DIC secondary to giant hemangiomas and purpura fulminans has been reported in neonates. Well-documented cases of DIC have been reported in infants with the acute respiratory distress syndrome.<sup>171,203,264</sup> The incidence of DIC in association with this syndrome and the importance of neonatal hypoxia in general have yet to be established. Some findings suggesting DIC have been noted in infants with hemolytic disease of the newborn (Chapter 54), but detailed studies are lacking. Even less clear is the hypothe-

sized role of DIC in hyaline membrane disease.<sup>271</sup>

#### *Clinical Picture*

The major clinical features of DIC are bleeding, often of serious magnitude and abrupt onset, a variable element of shock that is often out of proportion to apparent blood loss, and symptoms of hypoperfusion of various vascular beds. Acute renal failure is commonly present, and thromboembolic manifestations are not uncommon.<sup>143,219</sup> Any of these features, or signs and symptoms of the underlying disorder, may predominate in a given case.

#### *Acute DIC*

Bleeding manifestations of virtually every kind have been described, and evolve rapidly in the patient with acute DIC. The first evidence often pertains to the underlying disorder. In abruptio placentae, shock develops rapidly; vaginal bleeding may be minimal or absent for a time and bears little relationship to the extent of abruption.<sup>239</sup> In cases that develop following surgical procedures, alarming hemorrhage may develop around drains and tracheostomies, and large accumulations of blood may be concealed in serous cavities. Generalized ecchymoses, petechiae, and bleeding from previously intact venipuncture sites or around indwelling intravenous needles or catheters are present in many patients. Large, spreading, hemorrhagic skin lesions often are superimposed on familiar exanthems in patients with rickettsial and viral infections. In patients with meningococcemia, cutaneous hemorrhage may be striking. Bleeding from apparently normal gingivae, epistaxis, gastrointestinal bleeding, and hematuria are very common.

In some forms of DIC, bleeding may not be immediately apparent, e.g., in septicemia due to gram-negative bacilli. In septic abortion, shock and acute renal failure often dominate the picture, as does evidence of acute cor pulmonale in amniotic fluid embolism and fat embolism.

**PURPURA FULMINANS.** In this disorder, hemorrhagic manifestations develop several days after an acute infection, usually of streptococcal origin. The most common manifestations are symmetrical ecchymoses of the lower extremities and buttocks, sharply circumscribed infarcts of the skin ("purpura necrotica") and genitalia, and gangrene of the extremities ("purpura gangrenosa") that often involves the digits symmetrically.<sup>148</sup> Petchiae are reportedly rare. Fever and prostration usually are marked, but visceral lesions, including renal involvement, are relatively uncommon.

### **Chronic DIC**

Superficial but extensive ecchymoses of the extremities, often without petechiae, may develop intermittently or may persist for weeks or months. Recurrent episodes of epistaxis or more serious internal mucosal bleeding may punctuate the course. Thromboembolic phenomena are very common, and frequently are the presenting complaints. Thrombophlebitis may develop in unusual sites, eg, the axillary vein. The picture of thrombophlebitis that repeatedly recurs after anticoagulant therapy has been stopped is not uncommon. More serious hemorrhagic manifestations may develop as the underlying disease progresses, or may arise with dramatic suddenness following surgical procedures, eg, prostatectomy. Acute DIC may be heralded by further thrombophlebitis or pulmonary emboli. In some patients, signs suggesting vascular obstruction may develop with little bleeding, eg, impairment of renal function, confusion, transitory neurologic syndromes, or repeated episodes of cerebral thrombosis. In intrauterine fetal death, bleeding is seldom serious, but progressive impairment of renal function is common.

### **Laboratory Diagnosis**

The laboratory findings in DIC are summarized in Table 38-2. Contrary to what is commonly assumed, they are quite variable. The plasma fibrinogen level, thrombin time,

platelet count and estimates of FDP, and, to a lesser extent, the PTT and prothrombin time are the cornerstones upon which the diagnosis of DIC is based. These simple tests should always be obtained first. Additional information may confirm but seldom refutes the diagnosis of DIC if typical abnormalities are demonstrated by these tests. A possible exception to this generalization is DIC in neonates or infants, in whom results of one-stage screening tests are more variable than in adults (page 1067).<sup>171</sup> Laboratory data change with remarkable rapidity in DIC, and in doubtful cases it is often important to repeat the simple tests at frequent intervals, even every four to eight hours.

Laboratory data must be interpreted with unusual caution in DIC. Levels of platelets and various coagulation factors, fibrinogen and factor VIII in particular, may be markedly elevated in most of the conditions associated with DIC, including pregnancy. Thus, a fibrinogen level of 200 mg/dl, although within the normal range determined in healthy subjects, may represent a marked drop in a patient whose baseline level was 800 mg/dl.

### **Basic Blood Examinations**

In DIC, routine hematologic tests may reveal evidence of acute bleeding, accelerated red cell destruction, or signs of the underlying disease. Examination of the blood smear may reveal schistocytes, and more subtle evidence of intravascular hemolysis is commonly found, eg, increased serum levels of lactic acid dehydrogenase, decreased haptoglobin. Rarely, massive intravascular hemolysis with hemoglobinemia and hemoglobinuria may be present.<sup>253</sup>

Thrombocytopenia is an early and consistent sign of DIC; it is difficult to entertain this diagnosis in the presence of a persistently normal platelet count. Platelet counts in the range of 50 to 100 × 10<sup>9</sup>/l are the usual finding, but severe thrombocytopenia may be present. In patients with DIC due to gram-negative septicemia, thrombocytopenia may develop before coagulation abnormalities ap-

pear,<sup>123</sup> whereas in those with DIC due to infection with gram-positive bacteria, and in those having other forms of DIC, the platelet count often falls simultaneously with the fibrinogen level. The bleeding time is usually prolonged even when the platelet count is only moderately depressed, presumably as the result of the impairment of platelet function by FDP.

### *The Coagulation Defect*

The PTT, prothrombin time, and thrombin time are prolonged in most patients with acute DIC. The thrombin time usually is not normalized by the admixture of the patient's plasma with an equal volume of normal plasma. In an occasional patient, "late" or small FDP predominate and the thrombin time may be normal. Early in the course of acute DIC, and in chronic DIC, the PTT may be normal, or even shorter than normal as the result of the procoagulant effects of activated coagulation factors. The coagulation time of whole blood varies widely; it may be shortened, normal, or prolonged. Truly incoagulable blood is not common, but the clot that forms in the presence of hypofibrinogenemia may be small, friable, and "wispy," and may be trapped within sedimented erythrocytes. This artifact (the "fall-out" phenomenon) often is attributed to fibrinolysis. Much has been written about the importance of "observing the clot,"<sup>239</sup> but this procedure seldom provides definitive information and may seriously mislead the inexperienced observer.

There are few patients in whom the process of DIC can be followed from its inception and specific assays for various coagulation factors obtained at the time of diagnosis reveal a variable and rapidly changing picture. The plasma levels of fibrinogen and of factors V, VIII, and XIII usually are significantly depressed; fibrinogen and factor V are the most consistently affected.<sup>219</sup> The level of factor X may be lower than that of other "stable" factors (factors VII, IX, and XI), which usually are present in normal amounts.<sup>219</sup> In many patients, particularly

those with abruptio placentae, normal prothrombin levels are maintained,<sup>219,273</sup> but marked hypoprothrombinemia often is present in those with septic DIC.<sup>141</sup> In patients with chronic DIC, prothrombin deficiency is unusual, whereas significant deficiencies of factor X often are present.<sup>219</sup> Marked deficiencies of all factors have been described in some patients.

The levels of factors VIII, IX, and XI as determined by one-stage assays may fluctuate widely as the result of the presence of activated factors,<sup>224a</sup> eg, thrombin,<sup>236</sup> factor Xa.<sup>141</sup> This problem is minimized in two-stage assays employing the thromboplastin generation test (page 1057).<sup>219,229</sup> In many patients, the levels of coagulation factors tend to "overshoot" after repletion, and in some a cycle of depletion and "overshoot" gives rise to very confusing data.<sup>115,212,236</sup> Fibrinogen levels may be overestimated by turbidimetric and certain precipitation methods, and, as the consequence of the inhibitory effects of FDP, the fibrinogen titer and the serial thrombin time may be prolonged out of proportion to the fibrinogen level.

### *Tests for Fibrinolysis and FDP*

Both the whole blood clot lysis time and the euglobulin clot lysis time are normal in most patients with DIC. In the presence of severe hypofibrinogenemia, the euglobulin clot may be too small to allow accurate measurement of lysis time. Moreover, even when plasminogen activators are present in high concentrations, the euglobulin lysis time may be normal or prolonged due to plasminogen depletion. Techniques in which an exogenous source of fibrinogen and plasminogen is provided circumvent these difficulties.<sup>11,113</sup>

If FDP levels are estimated by means of the Fi reagent, titers of 1:8 or less can be regarded as normal,<sup>28</sup> and, in most patients with DIC, titers of 1:50 or higher will be found. With quantitative methods, such as staphylococcal clumping<sup>173</sup> or red cell bemaagglutination inhibition,<sup>28</sup> normal levels of FDP, expressed as "fibrinogen equivalents," range up to 8  $\mu\text{g}/\text{ml}$ . In most patients with



DIC, levels of 25  $\mu\text{g/ml}$  or higher will be found.<sup>219</sup> All methods are more sensitive to large or "early" FDP. These fragments, particularly fragment  $X_2$ ,<sup>236</sup> may retain thrombin-binding sites or may form a complex with fibrinogen, and consequently may be removed during the preparation of serum for FDP tests. This is one explanation for the absence of FDP in an occasional patient with otherwise typical DIC. Tests for FDP and the euglobulin lysis time are unreliable when performed on cord blood.

"Paracoagulation" techniques are simple to perform but are much less specific than tests for FDP.<sup>286a</sup> The ethanol gelation test<sup>133</sup> is much less sensitive than tests for FDP and frequently is negative in DIC.<sup>164</sup> To the contrary, results of protamine gelation tests<sup>227,257</sup> usually are positive, but abnormal results are obtained in numerous other disorders, including many that frequently are associated with DIC.<sup>164</sup> Such tests are valuable as "broad-spectrum" screening procedures. "Cryofibrinogen" may be present,<sup>198,253,263</sup> and should not be confused with small insoluble clots that are frequently found in plasma samples.

### Miscellaneous

The survival time of <sup>125</sup>I-labeled fibrinogen<sup>117</sup> and the rate of incorporation of <sup>14</sup>C-labeled glycine ethyl ester into soluble "circulating fibrin"<sup>182</sup> are exceptionally sensitive indicators of DIC, and may be significantly abnormal even in patients with normal levels of FDP. The third component of complement is decreased in many patients with DIC.<sup>163,279</sup>

### Differential Diagnosis

There are relatively few causes of serious hemorrhage of abrupt onset in a patient who was previously free of bleeding, and there are very few acquired disorders that give rise to significant coagulation abnormalities in which the cause is not obvious. Thus, the syndrome of DIC is seldom difficult to recognize. Most

often, problems arise when the diagnosis simply is not considered, or, in the chronic forms, when the underlying process may be masked by features of the basic disease or by thromboembolic complications. In patients with carcinoma and chronic DIC, laboratory evidence of microangiopathic hemolytic anemia may be the major finding.<sup>117,180,259</sup> In promyelocytic leukemia, intravascular coagulation may proceed at a very slow rate,<sup>162</sup> but thrombosis is a common complication.<sup>310</sup>

Two disorders, however, produce laboratory abnormalities that resemble DIC, ie, severe liver disease, which is very common, and fibrinogenolysis or "pathologic" fibrinolysis, which is very rare (Table 38-2).

In *fibrinogenolysis* (page 1224), the presence of hypofibrinogenemia, FDP, abnormalities in the PTT, prothrombin time and thrombin time, and deficiencies of factors V and VIII may be noted, but the platelet count usually is normal, and the euglobulin lysis time is markedly and persistently shortened, often in association with plasminemia. Schistocytes are not seen in association with fibrinogenolysis, unless they are the result of an underlying disorder. Hypoprothrombinemia and deficiencies of stable coagulation factors VII, IX, X, and XI are very rare in fibrinogenolysis, and the results of paracoagulation tests are normal.<sup>117</sup>

In *liver disease*, coagulation abnormalities and thrombocytopenia may originate from many pathologic processes (page 1205). Chronic or intermittent fibrinogenolysis with high levels of FDP is commonly present, particularly in cirrhosis.<sup>11</sup> In such patients, the exclusion of DIC may be very difficult. In contrast to DIC, factor VIII levels are usually elevated in severe liver disease, and the levels of factors VII and IX usually are low.

DIC may be confused with various *microangiopathic hemolytic anemias* (Chapter 28), particularly with thrombotic thrombocytopenic purpura and the hemolytic uremic syndrome, disorders in which the clinical picture may resemble that of DIC in many respects. High levels of FDP may be encountered in the microangiopathic hemolytic anemias, but

coagulation abnormalities are not commonly present.<sup>196,236</sup> Many other disorders may produce significant elevations of FDP, eg, pulmonary embolism, chronic renal disease.

Moderate thrombocytopenia is a common consequence of the use of extracorporeal circulatory devices, and marked coagulation abnormalities often are noted immediately following their use because of the presence of residual amounts of heparin. Heparin levels may also rise several hours later (the "rebound" phenomenon).<sup>166,237</sup> In such patients, the presence of thrombocytopenia together with the effects of heparin may result in a state that will be confused with DIC. Similar difficulties in differentiation may arise following hemodialysis. Even the small amounts of heparin required to irrigate indwelling catheters are a common cause of "pseudo-DIC."

## Treatment

### Anticoagulants

The rationale for the use of heparin in the treatment of DIC would seem clear-cut. This drug is a potent and specific antagonist of thrombin and also inhibits various steps in coagulation (page 1239), and thus can rapidly neutralize free thrombin and retard or stop its further formation. The therapeutic efficacy of heparin is unquestionable in some animal models of DIC, but assessment of its effectiveness in man has proved more difficult. Heparin has been given concomitantly with numerous other agents to most patients with DIC. In those with acute forms of DIC in which the activating stimulus is transitory, eg, abruptio placentae, spontaneous cure is the rule. It should be remembered that heparin does not alter the basic disease process that is responsible for intravascular coagulation. In view of the complexity of DIC it is apparent that inhibition of coagulation alters only one facet, albeit a fundamental one, of the pathophysiologic cycle.

In chronic DIC the results of heparin therapy usually are favorable and may be dramatic.<sup>115,130,179,212</sup> In most subjects, heparin

would not be expected to alter ultimate mortality because of the nature of the underlying diseases, but, in the majority, this drug reduces the severity of bleeding and produces parallel improvement in laboratory abnormalities. The restoration of normal levels of the coagulation factors occurs roughly in inverse proportion to their biologic half-lives, but there are numerous exceptions to this rule. The laboratory picture of the "hypercoagulable state" frequently is seen following effective heparin therapy.<sup>115,229,242</sup> Fibrinolysis, if present, usually disappears following the administration of heparin, often before the coagulation defect has been alleviated.<sup>229</sup>

In acute DIC, particularly that associated with septicemia, results have been less encouraging.<sup>183</sup> In one series,<sup>141</sup> the alleviation of septic shock appeared to be more important in the ultimate prognosis than did correction of the coagulation abnormalities. Nevertheless, extant data do not justify the omission of heparin therapy in the treatment of DIC due to septicemia, even though such therapy has clearly been effective in only a minority of these patients. The administration of heparin together with adrenal corticosteroids has proved effective in a few patients with purpura fulminans.<sup>108,111,174</sup>

The apparent paradox of administering anticoagulants to a patient with a serious bleeding disorder has been emphasized repeatedly, but there is little evidence that heparin therapy is associated with unusual risk in DIC. Most workers have used standard dosage regimens (Table 39-1, page 1240), but some advise a reduction of the dosage in the face of marked thrombocytopenia.<sup>169</sup> Good therapeutic responses have been documented even with doses that do not significantly prolong the coagulation time.<sup>115</sup> Intermittent intravenous therapy (5,000 to 10,000 U/kg every four to six hours) or a continuous intravenous drip (1000 to 1500 U/hour) usually is preferred to administration by the intramuscular or subcutaneous route. Care should be used in patients with renal insufficiency and in those with severe hepatic disease, but neither condition contraindicates the use of heparin.

Laboratory control of heparin therapy is difficult in DIC. A rough estimate of the heparin level can be obtained from the coagulation time. Except in patients with severe hypofibrinogenemia, an adequate end point can usually be obtained. The results of the PTT are frequently erratic in the presence of DIC.

Heparin therapy is seldom required in patients with some forms of DIC. DIC that develops during or following extracorporeal circulation and other major surgical procedures often is transient. In abruptio placentae, DIC is rapidly reversible if the uterus is evacuated promptly; blood loss may be a major factor in the production of shock, and heparin may increase the risk of hemorrhage.<sup>239,250</sup> Heparin is seldom required in the management of chronic DIC associated with intrauterine fetal death, unless the dead fetus is carried for more than a month; then heparin should be administered only to patients who have a falling fibrinogen level and significant bleeding.<sup>239</sup>

There is no evidence that coumarin anticoagulants, except in unusually high and toxic doses,<sup>115</sup> are effective in the management of DIC, even in its chronic forms, and there are several reports detailing the recurrence of previously controlled DIC when heparin was replaced by coumarins.<sup>115</sup> The effects of dextran<sup>130</sup> and of inhibitors of platelet function such as aspirin have not been adequately evaluated.

#### *Replacement of Platelets and Coagulation Factors*

The value of replacement therapy in DIC is limited, first, because the deficiency of most coagulation factors is seldom marked, and, secondly, because the most significant factor in the production of bleeding probably is the presence of large amounts of FDP.<sup>143</sup> The value of fibrinogen administration is debatable. In theory, the infusion of fibrinogen in the presence of active DIC "adds fuel to the fire." Thromboembolic complications have developed in some patients following fibrinogen administration,<sup>212,236</sup> but, in a much

larger number, fibrinogen produced no apparent adverse effects, and the risk of thromboembolism is greatly minimized if the patients are receiving heparin. It is probable that fibrinogen administration should be restricted to the occasional patient with marked hypofibrinogenemia and significant bleeding, in whom DIC is self-limited or has been controlled by heparin, eg, in intrauterine fetal death, prior to surgical intervention. Fibrinogen is frequently administered to women with abruptio placentae following evacuation of the uterus,<sup>239</sup> particularly if immediate surgical treatment is required. Even this indication has been disputed, however.<sup>273</sup> An additional argument against the use of purified fibrinogen is the risk of hepatitis, which may develop in as many as 20% of patients.

Because platelets are repleted slowly following a major episode of DIC, platelet transfusions may be helpful in patients with severe thrombocytopenia. The administration of concentrates of specific coagulation factors is rarely indicated.<sup>176</sup>

#### *Miscellaneous*

Treatment of shock should be prompt and energetic in all patients with DIC. There is no evidence that transfusions of whole blood or plasma promote thromboembolic complications, and these components should be given promptly if indicated. There is experimental<sup>223</sup> and some clinical evidence<sup>127</sup> that corticosteroids accelerate DIC. Nevertheless, these hormones are commonly given to patients with purpura fulminans and to those with meningococcemia.

In theory, epsilon aminocaproic acid (EACA) and other antifibrinolytic agents (page 1226) remove the major antagonist of intravascular fibrin formation, and in several well-documented cases of DIC, their use has resulted in serious and even fatal thromboembolic complications.<sup>212,234</sup> The indiscriminate use of such drugs should be discouraged. EACA has been administered together with heparin<sup>187</sup> or after heparin in a few patients in whom DIC was associated with fibrinogenolysis. No serious adverse effects were

observed, but the therapeutic value of EACA was difficult to assess. Because of the potential risks, fibrinolytic enzyme inhibitors should be administered only to carefully selected patients; ie, those in whom DIC has resulted from a transitory stimulus or has been arrested by heparin administration, and in whom fibrinogenolysis, hypofibrinogenemia, and adequate renal function have been clearly documented.

It is possible that the therapeutic benefits of antifibrinolytic drugs outweigh potential adverse thromboembolic effects in many other forms of DIC, but this question cannot be answered with available data. The therapeutic use of EACA in fibrinogenolysis and appropriate dosage schedules are discussed on page 1226.

Agents such as *streptokinase* and *urokinase* activate the endogenous fibrinolytic enzyme system and have been used in some patients with DIC in an attempt to accelerate recanalization of thrombosed vessels after the acute process has been arrested. Preliminary results have ranged from equivocal to encouraging. It should be emphasized that the therapeutic margin between effective thrombolysis and fibrinogenolysis is perilously narrow.<sup>212</sup>

Exchange transfusion has been tried with some success in a few patients with DIC.<sup>264</sup>

## Pathology

The deposition of fibrin in small vessels represents the ultimate result of DIC. In many patients, fibrin can be formed and lysed without significant vascular occlusion. Indeed, in many autopsied subjects, fibrin thrombi were absent or were demonstrated only with special stains or by electron microscopy. This state may result from agonal lysis, or from deposition of thin films of fibrin on the vast endothelial surface and on the erythrocytes. The localization of fibrin thrombi varies somewhat with the cause of DIC. The usual distribution of lesions in the gut, pancreas, adrenals, brain, liver, skin, and kidneys has been well documented.<sup>208</sup> Renal lesions range from patchy tubular necrosis to

massive bilateral cortical necrosis.<sup>248</sup> Non-thrombotic endocarditis and pulmonary hyaline membranes have been found in some patients.<sup>177</sup>

## Fibrinogenolysis

The term "*pathologic fibrinolysis*" has been used indiscriminately for many years to refer to virtually any situation in which in vitro evidence of fibrinolysis was associated with bleeding. It seems probable, in retrospect, that in many cases fibrinolysis was secondary to DIC, and that in others it represented an essentially physiologic response to anoxia, shock, or stress.<sup>9</sup> It is improbable that fibrinolysis per se ever produces bleeding, although the lysis of hemostatically functional fibrin plugs may aggravate bleeding from other causes,<sup>211</sup> and may explain many cases of postoperative "oozing."<sup>185,288</sup> In *fibrinogenolysis* the proteolytic destruction of fibrinogen and other proteins occurs in the general circulation, and severe bleeding may develop. The pathophysiology of fibrinogenolysis remains unclear and in many instances it is probable that this process also represents a disproportionate or "inappropriate" response to underlying DIC, eg, in amniotic fluid embolism. Whether or not DIC is the common denominator in all cases of "pathologic fibrinolysis" and fibrinogenolysis is a question of great practical importance which cannot be answered with certainty.<sup>155a</sup> Since FDP presumably are major factors in the production of bleeding in fibrinogenolysis as well as in DIC,<sup>141,262</sup> antifibrinolytic agents would seem therapeutically desirable. However, these drugs are hazardous in the presence of DIC. This therapeutic "paradox" is presently insoluble.

## Etiology

It is questionable whether fibrinogenolysis ever develops in the absence of an underlying disease,<sup>9,219</sup> but this process may complicate various disorders, among which severe liver disease is by far the most common (page 1205). Fibrinogenolysis was the predomi-

nating laboratory feature in several patients with disseminated neoplasms,<sup>244,276</sup> and in others following extensive surgical operations,<sup>266</sup> particularly procedures involving the lung<sup>105,268</sup> and those performed for the correction of cyanotic congenital heart disease. Fibrinogenolysis has been reported in other disorders<sup>112</sup> including several that involve the hematopoietic system, eg, polycythemia vera,<sup>309</sup> acute and chronic leukemia,<sup>188</sup> and various lymphomas.<sup>62,132</sup>

### Pathophysiology

Fibrinogenolysis is a consequence of the activation of plasmin within the general circulation (*plasminemia*) (page 434). Potent plasma inhibitors (antiplasmins) normally neutralize free plasmin rapidly (Fig. 10-7, page 433), with the result that the proteolytic effects of this enzyme normally are restricted to fibrin, which is presumably its physiologic substrate. Fibrinogenolysis occurs only when the capacity of the antiplasmins is exceeded.

The proteolytic action of plasmin is quite nonspecific. In addition to fibrin and fibrinogen, this enzyme may degrade other coagulation factors<sup>267</sup> as well as a wide variety of other plasma proteins, eg, complement, various hormones.<sup>235</sup> Free plasmin may activate bradykinin, a phenomenon that may underlie the marked hypotension present in some patients with fibrinogenolysis.<sup>188</sup> "Pathologic proteolysis"<sup>262</sup> is thus an appropriate synonym for fibrinogenolysis.

Fibrinogenolysis is activated by mechanisms that are remarkably similar to those that initiate DIC. Thus, tumor tissues contain large amounts of plasminogen activators in addition to thromboplastins.<sup>105</sup> The sudden entry of such tissue extracts into the circulation may activate most of the circulating plasminogen instantaneously. Slower but more continuous "autoinfusion" presumably is involved in most cases, and it is probable that intravascular coagulation coexists in many. A similar process presumably explains the transient fibrinogenolysis that occurs following major surgical procedures.<sup>288</sup>

Hypoxia and hypoperfusion may lead to plasminogen activation, and, in an occasional patient, to fibrinogenolysis. However, in many of these patients, bleeding was minimal, and when present could not be clearly related to the presence of fibrinogenolysis. It is probable that, in these patients, fibrinogenolysis is a nonspecific essentially physiologic response. A similar conclusion has been reached regarding the majority of cases of "pathologic fibrinolysis" which develop following extracorporeal circulation.<sup>124,237,298</sup> Fibrinogenolysis may result from therapy with thrombolytic enzymes, as discussed on page 1247.

### Clinical and Laboratory Diagnosis

The clinical picture in most reported cases of fibrinogenolysis is quite similar to that present in DIC. The usual laboratory findings are summarized in Table 38-2 and are discussed on page 1221. Hypofibrinogenemia is seldom marked, and when found may in part represent an artifact due to in vitro lysis during processing of the blood sample. The PTT, prothrombin time, and thrombin time may be markedly prolonged, due mainly to the anticoagulant effects of FDP. Moderate concentrations of EACA ( $4 \times 10^{-4}M$ )<sup>288</sup> inhibit plasminogen activators but not free plasmin. Thus, the euglobulin lysis time, which in fibrinogenolysis invariably is shortened, is unaffected by the addition of EACA if free plasmin is present. Fibrinolysis in heated fibrin plates<sup>113</sup> also measures free plasmin, since plasminogen activators are thermolabile. Among the coagulation factors, factors V and VIII are the most sensitive to the proteolytic action of plasmin. The plasma levels of other factors, including some that are degraded by plasmin in vitro,<sup>267</sup> usually are normal in fibrinogenolysis, eg, factors VII and IX. Factor XIII deficiency was present in some patients.<sup>116</sup>

Presently available techniques for measuring FDP do not discriminate between fibrinogen degradation products and fibrin degradation products, and both produce similar

impairment of hemostatic functions<sup>153,220</sup> (Fig. 10-8, page 437). In the absence of intravascular coagulation, paracoagulable complexes involving fibrin monomers do not form in plasma; thus, the plasma protamine gelation test is negative in fibrinogenolysis.<sup>223</sup> The serum paracoagulation test is positive because of the presence of FDP. Assays for plasminogen and for the various inhibitors of the fibrinolytic enzyme system are quite time-consuming, and are seldom of critical diagnostic importance. Such special techniques may be useful in monitoring therapy with thrombolytic agents (page 1247).

### Treatment

*Epsilon aminocaproic acid* (EACA) is a specific and potent inhibitor of fibrinolysis and fibrinogenolysis. In low concentrations, it inhibits plasminogen activation competitively, and in high concentrations ( $> 0.06$  M) it inhibits plasmin directly in a noncompetitive manner.<sup>106</sup> The clinical effectiveness of this drug is dramatic in carefully selected patients.<sup>228</sup> Complete inhibition of fibrinolysis requires relatively large doses. EACA should be administered intravenously (0.1 g/kg every six hours)<sup>228</sup> if bleeding is severe. The drug is rapidly absorbed after oral administration, and 1 g/hour following a 5-g loading dose is quite effective. The total dose of this drug should not exceed 30 g in a 24-hour period. Newly developed antifibrinolytic agents<sup>110,147</sup> may be even more effective than EACA, but require more thorough evaluation.

Although the effectiveness of EACA as an antifibrinolytic agent is unquestionable, the indications for its use remain vague and controversial. In view of the difficulty of excluding underlying DIC, it has been recommended that "in doubtful cases" EACA should be used together with heparin or following heparin therapy.<sup>228</sup> Unfortunately, information regarding the efficacy of such concomitant or sequential therapy is very limited.<sup>147</sup>

## Miscellaneous Acquired Coagulation Disorders

Postoperative bleeding is a common and serious problem in patients with polycythemia vera<sup>209</sup> and in those with cyanotic congenital heart disease,<sup>62</sup> but the cause remains poorly understood in both disorders. In patients with *polycythemia vera*, various coagulation abnormalities have been reported, the most common being prolongation of the prothrombin time and deficiency of factor V.<sup>309</sup> Fibrinolysis, thrombocytosis, and platelet dysfunction have been prominent in other patients with this condition. The cause and relative importance of all of these findings remain uncertain. Even more confusing are data pertaining to *cyanotic congenital heart disease*.<sup>300</sup> Reports emphasizing the importance of increased fibrinolytic activity<sup>293,296</sup> appear to be in direct conflict with other accounts in which DIC was implicated.<sup>299,298,301</sup> Still other studies have revealed thrombocytopenia without significant coagulation abnormalities.<sup>302,310</sup>

Minor coagulation abnormalities have been described in association with myelofibrosis, Hodgkin's disease, and other lymphoreticular disorders.<sup>62,158,230</sup> The significance of these findings is difficult to assess, since, in all of these disorders, laboratory abnormalities correlate poorly with the severity of bleeding.

A curious phenomenon, as yet to be explained, is the development of deficiencies of a single factor during the course of an acquired disorder, eg, severe acquired hypoprothrombinemia.<sup>303</sup> Such isolated deficiency of factor X has been well documented in amyloidosis.<sup>99,291,301,308</sup> Deficiency of factors IX<sup>307</sup> and XII<sup>297</sup> has been reported in the nephrotic syndrome, presumably as the result of massive protein loss in the urine. In one patient with Sheehan's syndrome, a selective deficiency of factor IX responded to treatment with corticosteroids and thyroid.<sup>294</sup> An acquired syndrome resembling von Willebrand's disease also has been described.<sup>299a</sup> Mild deficiencies of factors V and VIII may be associated with *massive blood transfu-*

sions,<sup>299,306,311</sup> which also may produce citrate intoxication,<sup>290</sup> and thrombocytopenia (page 1102). Minor coagulation abnormalities have been reported in uremia,<sup>305</sup> but the major cause of bleeding in the uremic patient is platelet dysfunction (page 1129). Many other acquired coagulation abnormalities reported in the older literature are now presumed to have resulted from DIC.

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## Thrombosis and Antithrombotic Therapy

### Pathophysiology of Thrombosis

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homeostatic control mechanisms may be of pathophysiologic importance in thrombosis. The present discussion is perforce limited to the nature and importance of these alterations, and the mechanisms by which antithrombotic agents affect the platelets and the coagulation factors. No attempt will be made to provide details concerning the pharmacology of the many anticoagulant drugs, or to discuss the pathophysiology and management of specific thromboembolic complications. For an introduction to the vast body of information concerning thrombosis, the reader is referred to several excellent reviews,<sup>42,47,118,128,131,136a,179,196</sup> and books,<sup>83,100,108,139,168,171</sup>

THE term "thrombosis" refers to the formation, from constituents of the blood, of an abnormal mass within the vascular system of a living animal. Thrombosis thus involves the interplay of vascular, cellular, and humoral factors within a flowing stream of blood. It is a kinetic process to be distinguished from the static phenomenon of blood coagulation.

Thrombosis of the veins and arteries, together with complicating embolic phenomena, is perhaps the most important cause of sickness and death in the developed countries of the world at the present time.<sup>82</sup> It is of far greater overall clinical importance than all of the hemorrhagic disorders combined.

There is preliminary evidence that alterations in the hemostatic apparatus and its

### Pathophysiology of Thrombosis

That abnormalities of the vessel wall, alterations of blood flow, and changes in the composition of the blood are the major factors in the pathophysiology of thrombosis was well recognized in the 19th century. The hematologic aspects of thrombosis may still be considered in terms of these three factors, which usually are known as the *triad of Virchow* (Fig. 39-1).

#### Role of the Vessel Wall

*Arterial thrombosis* often is the result of a process that damages the vessel wall, eg, ath-

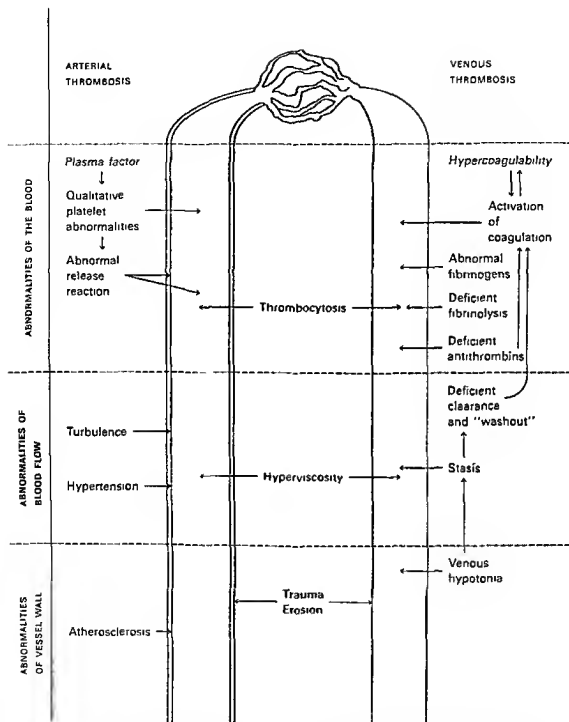


Fig 39-1 Pathophysiology of thrombosis. Shown are some of the factors implicated in the pathophysiology of arterial thrombosis (left) and venous thrombosis (right). The processes illustrated are highly theoretical.

erosclerosis. It commonly begins with the formation of a tūdus of platelets and fibrin on the vascular surface.<sup>179</sup> This process may be initiated by ADP or a similar chemical mediator that is released from injured vessels,<sup>12,95</sup> or it may result from exposure of collagen fibers in the vessel wall.<sup>7,179</sup> This initiates the processes of platelet adhesion, aggregation, and thrombus formation in much the same manner as it leads to the formation of a normal hemostatic plug (Chapter 9). Neither of these hypotheses explains how arterial thrombi propagate far beyond the site of injured endothelium,<sup>82</sup> and in many forms of arterial thrombosis the pathophysiology is unclear.

In *venous thrombosis*, the vessel wall usually is histologically normal, and factors extrinsic to the vessel appear to have the major pathophysiologic role. An exception to this generalization is direct trauma to or erosion of veins. There is some evidence that fibrinolytic activity in the veins of the legs is subnormal, as compared to that in other veins.<sup>150</sup> A generalized reduction in venous tone may be an important pathophysiologic factor in venous thrombosis in pregnant women, and in women taking oral contraceptives.<sup>72,202</sup>

### Role of Abnormalities of Blood Flow

*Arterial thrombosis* initially occurs under conditions of rapid blood flow, and arterial thrombi are presumably non-occlusive for a time. They usually are composed of a tightly coherent mass of platelets, which contains small amounts of fibrin and a few erythrocytes and leukocytes. These are the classic *white thrombi*, which resemble, in many respects, the normal hemostatic plug (page 396). As arterial thrombi enlarge, progressive or intermittent deposition of new layers of platelets and fibrin produces the characteristic lines of Zahn, and partial or complete obstruction of blood flow may produce a "tail" of red thrombus. Abnormalities of blood flow are of uncertain pathophysiologic significance in arterial thrombosis, although turbulent blood flow and hyperviscosity may be con-

tributory factors in certain forms.<sup>134</sup> The most serious consequences of arterial thrombosis are those caused by vascular occlusion.

*Venous thrombosis* develops under conditions of slow blood flow, and is favored by further retardation and stagnation of flow.<sup>196</sup> Venous thrombi are composed of large amounts of fibrin containing numerous erythrocytes. In this loose, friable mass (*the red thrombus*) the platelets and leukocytes are enmeshed in random fashion. Venous thrombi resemble blood clots formed in vitro; they usually produce significant obstruction to blood flow from the outset, but their most serious consequences are those of embolization.

Studies of clots formed in a thromboviscometer at varying rates of shear,<sup>48</sup> and of serum-induced thrombi in animals,<sup>198</sup> suggest that the differences in the structure of venous and arterial thrombi may be mainly the result of the velocity of blood flow. The many and very complicated *rheologic factors* that may be involved in thrombosis have been reviewed.<sup>71</sup>

The pathophysiologic role of stasis in venous thrombosis is well recognized.<sup>82,184,196</sup> It has been suggested that this is mediated by impairment of mechanisms which normally remove activated coagulation factors from the circulating blood; ie "washout"<sup>45</sup> (page 431), and hepatic clearance (page 432). Additional factors must be involved, however, since marked venous stasis occurs daily in normal persons; eg during dependency of the legs. These additional factors presumably reside in the coagulation mechanism, as discussed below.

### Role of Alterations in the Blood

The role that abnormalities of the hemostatic apparatus play in the pathophysiology of thrombosis remains very poorly understood. Many techniques originally devised for testing the hemostatic functions of platelets and coagulation factors appear to be poorly suited to the study of thrombosis. Moreover, with very few exceptions, thrombosis is a

natural occurrence only in man.<sup>60</sup> As a consequence, some animal models may prove to be irrelevant to the study of thrombosis in man.<sup>47,82</sup>

### Platelets

Thrombosis is a significant, if not the major, clinical feature of certain disorders associated with *thrombocytosis* (page 1103). The pathophysiology of thrombosis in these disorders is obscure. It is probable that an as yet undetermined qualitative abnormality of the platelets is involved. The importance of increased platelet numbers per se is uncertain.<sup>36,118</sup>

Attempts to demonstrate *qualitative abnormalities* of platelet function in patients or animals with various forms of thrombosis, and in patients with various disorders associated with an increased "risk" of thrombosis,<sup>100,193</sup> have resulted in a voluminous literature containing little clear-cut information.<sup>76,132,133</sup> Measurements of platelet aggregation,<sup>25,40,76,141,165</sup> platelet factor 3 (PF-3) availability,<sup>163</sup> and of various biochemical properties of the platelet<sup>139</sup> and the study of platelet thrombi artificially formed in the Chandler tube<sup>6,32,118</sup> have revealed minor or inconsistent abnormalities, most of which are probably nonspecific.<sup>82</sup>

Fatty acids induce platelet aggregation and the release reaction,<sup>92</sup> and are thrombogenic in some animals.<sup>38</sup> Hyperlipidemia in humans<sup>139</sup> and fat feeding in animals may produce an increase in "available" platelet factor 3.<sup>163</sup> In one study in animals, cholesterol feeding was thrombogenic only when thrombocytosis was induced by phlebotomy.<sup>26</sup> The pertinence of these observations to the pathophysiology of human thrombosis is unclear.

Studies of *platelet retention or adhesiveness* have suggested that the platelets may be abnormally "sticky" in patients with active thromboembolism<sup>76,88,98</sup> and in those with various disorders associated with an increased risk of thromboembolic complications, eg, obesity, smoking, hypertension, homocystinuria,<sup>123a</sup> diabetes.<sup>158,193</sup> Increased platelet

"adhesiveness" has been demonstrated more often with the methods of Wright<sup>203</sup> and Hellem<sup>86</sup> than with the Salzman technique.<sup>167</sup> There is evidence in some patients that increased platelet "stickiness" is the result, rather than the cause, of thromboembolic disease.<sup>51,88,97</sup> The abnormalities are slight, in any case, and many studies concerning platelet "stickiness" have been inadequately controlled.

Preliminary evidence would suggest that the *sensitivity of the platelet to ADP* may be significantly increased in various thromboembolic disorders.<sup>76</sup> This abnormality is best demonstrated by measurements of the electrophoretic mobility of the platelets,<sup>79,87</sup> and has been found in patients with ischemic heart disease,<sup>81</sup> persons having peripheral arterial disease,<sup>80</sup> in women who are pregnant and in those taking oral contraceptives,<sup>20</sup> and also in association with multiple sclerosis,<sup>19</sup> various acute illnesses,<sup>79</sup> and following surgical operations. The abnormal sensitivity to ADP apparently is not intrinsic to the platelets, but rather is the result of the presence of an abnormal plasma factor.<sup>82</sup> This factor may be a phospholipase capable of converting low-density lipoproteins, such as lysolecithin, into lecithin.<sup>14,77,128</sup> It is probable that increased platelet stickiness, described above, is an indirect result of increased sensitivity of the platelet to ADP.<sup>87</sup>

It has been suggested that platelet aggregates, formed in the circulating blood, may cause vascular damage and thrombosis following their lodgement on normal vessel walls.<sup>105,132,133</sup> In terms of this hypothesis, vascular injury may be mediated by substances extruded from platelets during the release reaction.<sup>136</sup> Such a process may also underlie the hypothesized role of the platelets in the pathogenesis of atherosclerosis.<sup>133,179</sup> This concept is provocative in that it reverses the traditional cause-effect sequence in the pathophysiology of arterial thrombosis. Nevertheless, it remains largely speculative. Serotonin and other vasoactive substances released from platelets adhering to emboli in the circulation presumably are the major



causes of the immediate vasomotor effects of pulmonary embolism.<sup>136</sup>

### Blood Coagulation

Over a hundred years ago, Troussseau called attention to the high incidence of recurrent and migratory thrombophlebitis in patients with certain carcinomas.<sup>29</sup> In such patients, and in those affected with many other disorders associated with a thromboembolic diathesis, abnormally rapid blood coagulation can be demonstrated by simple but crude tests, such as the determination of clotting time in siliconized tubes or the PTT.<sup>38</sup> The blood of such patients may be unusually resistant to the anticoagulant action of heparin, a phenomenon that may be measured by various heparin "resistance"<sup>191</sup> and "tolerance" tests.<sup>41,73</sup> High levels of various coagulation factors, particularly fibrinogen, factors V, VII, VIII, and X as well as thrombocytosis, have been well documented in the same disorders,<sup>29</sup> and also in pregnancy and in women taking oral contraceptives (page 421). Hereditary disorders associated with a thromboembolic diathesis and marked, selective increases in the plasma levels of factors V<sup>70</sup> and VIII<sup>151</sup> also have been described. These data have led to the concept that a propensity to thrombosis may be the result of *in vivo* hypercoagulability of the blood.

The determinants of the *hypercoagulable state* are poorly defined. There is no evidence that increases in the concentrations of a single coagulation factor, or even of several factors, are thrombogenic *per se*. High levels of several factors may result from processes not associated with an unusual incidence of thrombosis (page 421), such as hyperthyroidism, a disorder in which thrombosis is exceedingly rare.<sup>197</sup>

Studies of *serum-induced thrombosis* in animals<sup>190</sup> provide evidence that the major factor in the hypercoagulable state is low-grade activation of coagulation, rather than increased levels of various coagulation factors. In this animal model,<sup>45,199</sup> the infusion of

small amounts of serum produced the laboratory abnormalities of hypercoagulability. When stasis was superimposed, venous thrombosis consistently developed.<sup>45</sup> The activated forms of factors IX, X, and XI appear to be responsible for the thrombogenic effects of serum.<sup>45,199</sup>

A laboratory picture similar to that described in the hypercoagulable state may also be seen in patients with chronic diffuse intravascular coagulation (DIC) (page 1211). In such patients, the coagulation time and the PTT may be shortened because of the presence of traces of thrombin or other activated coagulation factors. These activated factors affect one-stage but not two-stage assays (page 1220). High levels of factors V and VIII may reflect the presence in the circulation of thrombin-activated forms of these factors (page 429). Pregnancy has been regarded as a "physiologic" form of chronic DIC by critical observers.<sup>111</sup> The effects of oral contraceptives appear to represent a lesser degree of the same process. These considerations, together with a large body of experimental data, lead to the hypothesis that the superficially different conditions of hypercoagulability and DIC may be fundamentally the same in many cases, differing only with respect to rate, magnitude, and the competence of homeostatic processes.

There is no direct evidence which establishes a cause-effect relationship between the laboratory abnormalities of hypercoagulability and the development of thrombosis in man, and it is probable, in many instances, that the laboratory features of the hypercoagulable state are the effects rather than the causes of thromboembolism. It has been suggested that a combination of stasis and low-grade activation of coagulation may be the major pathophysiologic factor in venous thrombosis in humans.<sup>42</sup> The mechanisms leading to activation of coagulation remain obscure.<sup>45</sup> There is little evidence that slow intravascular coagulation is a continuous physiologic process, as was once believed (page 439).

Tissue factor may form complexes with

factors VII and Xa (page 425). It has been suggested that such complexes, when formed in situ on an injured vascular surface, may persistently activate coagulation in the passing blood.<sup>200</sup> Increased levels of phospholipids may accelerate coagulation in vivo.<sup>157</sup> Fatty acids may induce contact activation<sup>121</sup> and may be thrombogenic in animals.<sup>21,38,39</sup> The significance of these phenomena in thrombosis in man is obscure. Minor abnormalities in the fibrinolytic enzyme system also have been demonstrated in association with various forms of thrombosis and some of the "risk" factors.<sup>125,191</sup> In several patients, inhibitors of fibrinolysis were associated with a life-long thromboembolic diathesis.<sup>21,133,137</sup> The activity of the fibrinolytic enzyme system is subnormal in pregnancy, as discussed elsewhere (page 436). The pathophysiologic significance of this observation is uncertain. Low levels of antithrombin III also have been demonstrated in a number of thrombotic disorders, but they may be the result, rather than the cause, of the pathologic state. A hereditary deficiency of antithrombin III was associated with a thromboembolic diathesis in some families.<sup>52</sup>

## Hematologic Methods for the Detection of Thromboembolic Disease

Attempts to develop blood tests that can "anticipate" the development of thrombosis or that can detect occult thromboembolic disease before it is recognized clinically have been largely fruitless.<sup>60,92</sup> None of the many alterations in platelet function and blood coagulation, described above, reliably indicates a "pre-thrombotic" state. Tests for the detection of these alterations may reveal abnormality following the development of thrombosis, but such findings are usually obtained after the disease has been recognized clinically.<sup>86</sup>

Studies of the turnover rate of isotopically labeled fibrinogen<sup>103</sup> and of products of fibrinogen catabolism and complexes formed therefrom (page 436) have yielded more

promising results. Levels of FDP, as determined by the usual techniques (page 1061), may be slightly elevated in the presence of active thrombosis, and usually are significantly elevated in the presence of pulmonary embolism.<sup>201</sup> A study of this family of protein fragments, by the determination of the gel filtration "pattern" of plasma,<sup>67</sup> suggests that a shift toward larger fragments occurs in hypercoagulability and thrombosis, and that the reverse is seen in fibrinolysis and fibrinogenolysis. This phenomenon apparently underlies the positive findings in para-coagulation tests that are frequently observed in association with these disorders.<sup>75</sup> These techniques are as yet imperfect,<sup>67</sup> and their utility in detecting the "pre-thrombotic" state is unproved. They may be valuable, however, in the recognition of occult thrombosis in sites where radiologic and isotopic techniques<sup>107</sup> are not helpful.

## Anticoagulant Therapy

In view of the minimal role played by blood coagulation in the pathogenesis of *arterial thrombosis*, it is not surprising that anticoagulants have proved to be of dubious value in the treatment of patients with this disorder.<sup>126</sup> Despite a few claims to the contrary, the therapeutic effectiveness of either heparin or coumarin-like drugs in arterial thrombosis appears to be attributable to limitation of further extension of the thrombus and the prevention of complicating venous thromboembolism.<sup>82,173,197</sup>

Anticoagulant therapy is clearly of benefit in *venous thrombosis*. Heparin is of proven effectiveness in the treatment of patients with thrombophlebitis and pulmonary embolism,<sup>126,184,197</sup> in the prevention of thromboembolic complications in bedridden patients, eg, those with fractured hips,<sup>109</sup> in carefully selected patients with cerebral embolism, and in some with diffuse intravascular coagulation (page 1222). Coumarin-like drugs are therapeutically useful in most of the patients in whom heparin is effective.<sup>126,184,197</sup> They are quite effective in the prevention of embolic complications in patients with artificial heart

valves and vascular prostheses.<sup>182</sup> The few carefully controlled studies that have been carried out suggest that these drugs are somewhat less effective than heparin; they are of limited value in DIC and in pulmonary embolism. However, they are widely used because they are cheaper than heparin, can be administered orally, and can be used on an outpatient basis.

### Heparin

Heparin is a highly acidic mucopolysaccharide composed of equal amounts of sulfated D-glucosamine and D-glucuronic acid, interlinked by sulfaminic bridges<sup>115</sup> (Table 39-1). Commercially available preparations are mixtures of heparins of several sizes, which range in molecular weight from 6000 to 20,000. Heparins prepared from the tissues of various animals have significantly different properties.<sup>192</sup> The differences between commercially available preparations, however, are of no certain practical significance.<sup>140</sup>

The unit of heparin is defined by biologic techniques in sheep.<sup>115</sup> USP heparin contains at least 120 U/mg; international standard heparin contains exactly 130 U/mg. Since the potency of individual preparations may range as high as 170 U/mg, caution is advised when this drug is prescribed by weight.

Heparin is active only when it is administered parenterally.<sup>115</sup> Its *in vivo* biodegradation proceeds as a first-order reaction with respect to blood heparin concentration.<sup>141</sup> It is degraded by heparinase, a hepatic enzyme. When administered in large doses, heparin appears in the urine in the form of a weakly anticoagulant complex termed "uroheparin."<sup>115</sup>

### Mechanism of Action

Heparin acts directly in the peripheral blood, and does not affect the hepatic biosynthesis or the plasma levels of any coagulation factor (Table 39-1). It combines with a variety of coagulation factors, presumably as the result of its highly negative charge, and thus may interfere with virtually any step in

coagulation.<sup>17,204</sup> In low concentrations, it inhibits the interactions between factors IXa, VIII, and PF-3 (reaction 4, Fig. 10-4, page 424), the "autocatalytic" actions of thrombin (reaction 13), and the action of factor Xa<sup>197</sup> (reaction 7). In higher concentrations, it inhibits the action of thrombin on fibrinogen (reaction 9).

All of the anticoagulant effects of heparin involve a preliminary interaction with plasma proteins, termed the *heparin cofactors*.<sup>1</sup> There is accumulating evidence that the major heparin cofactor is identical to antithrombin III.<sup>17,204</sup> This substance is the major "progressive" antithrombin of normal plasma, and also is an inhibitor of factor Xa.

Heparin inhibits certain platelet functions including the release of serotonin. This effect may be of therapeutic importance in minimizing the vasomotor sequelae of pulmonary embolism.<sup>164,185,186,198</sup> Heparin also may facilitate fibrinolysis, an effect that may be mediated by its lipolytic action,<sup>113</sup> to be discussed below. The physiologic or therapeutic significance of this phenomenon is uncertain.

There is no definite evidence that heparin normally is present in the blood, or that it acts as a physiologic anticoagulant. Very small amounts of this drug activate *plasma lipoprotein lipase* (heparin "clearing" factor), an enzyme involved in the lysis of triglycerides in chylomicra.<sup>115</sup> The physiologic importance of this action of heparin also is obscure. Several commonly used drugs interfere with the action of heparin (Table 39-1).<sup>149</sup> This anticoagulant does not cross the placenta.<sup>63</sup>

### Regimens

Although heparin has been used therapeutically for more than 30 years, there is no general agreement regarding preferred or optimal treatment schedules. The regimens summarized in Table 39-1 are those usually recommended for the treatment of patients with uncomplicated thrombosis.<sup>50,115,184</sup> It is generally agreed that higher doses than those summarized in Table 39-1 are needed early

Dr. B. I. Sharma, M.D.

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Table 39-1. Antithrombotic Agents

	<i>Heparin</i>	<i>Coumarins and Indanediones</i>	<i>Anacrod</i>	<i>Thrombolytic Agents</i>	<i>Inhibitors of Platelet Function</i>
Chemistry	Highly acidic mucopolysaccharide	Derivatives of 4 hydroxycoumarin or 1,3 indene done	Proteolytic enzyme semi purified from venom of Malayan pit viper	Proteolytic enzymes semi purified from bacterial products (streptokinase) and urine (urokinase)	Acetylsalicylic acid, dipyridamole, dextran, others
Mechanism of action	Inhibition, after interaction with cofactor of various coagulation reactions particularly reactions 4, 7, 9 and 13 (Fig 10-4, page 424)	Antagonism of biosynthetic action of vitamin K leading to deficiency and formation of abnormal forms of prothrombin and factors VII, IX, X (Fig 38-1, page 1203)	Proteolytic degradation of circulating fibrinogen leading to complete afibrinogenemia	Induction of fibrinolysis by endogenous activation of plasminogen (Fig 10-7, page 433)	Inhibition of platelet aggregation and the release reaction (Fig 9-6, page 370)
Site of action	Peripheral blood	Liver cell	Peripheral blood	Within or on the surface of a thrombus	Circulating platelet
Time of action	Immediate	After 12- to 48 hour lag	Immediate	Immediate, after neutralization of plasma inhibitors	Immediate
Usual route of administration	Parenteral	Oral	Intravenous	Intravenous	Oral
Therapeutic regimens	<p><i>Intermittent intravenous</i> 5 000-10 000 U every 4 hours</p> <p><i>Continuous intravenous</i> 5 000 U "loading" dose followed by infusion of 20 000-35 000 U in 1 l of fluid" per 24 hours</p> <p><i>Subcutaneous</i> 15 000-20 000 U of repository heparin every 12 hours</p>	<p><i>Warfarin</i>, 50 mg first day or 10-15 mg daily until prothrombin time is within therapeutic range thereafter 6 mg (1-12 mg) daily</p> <p><i>Bishydroxycoumarin</i>, 300 mg first day, 200 mg second day, thereafter, 75 mg (25-150 mg) daily</p>			

Laboratory control	Whole blood clotting time, activated PT†	Prothrombin time, or related test	Plasma fibrinogen level‡	Plasma fibrinogen level, thrombin time†	Epinephrine- or collagen-induced platelet aggregation: no laboratory control ordinarily required
Interfering substances	Antagonists (tetracyclines, antihistamines, digitalis, nicotine, ascorbic acid)	Antagonists (barbiturates, carbamazepine, cholestyramine, ethchlorvynol, glutethimide, griseofulvin, heptabarbital, oral contraceptives)	Incompletely studied	Incompletely studied	Incompletely studied
		Potentiators (anabolic steroids, chloral hydrate, chloramphenicol, clofibrate, dextrothyroxine, disulfiram (Antabuse), glucagon, mefenamic acid, neomycin, oxyphenbutazone, phenylbutazone, phytate, quindine, salicylates, some sulfonamides, ticlofos)			
Pharmacologic antidote	Protamine	Vitamin K	Specific antivenom	EACA, tranexamic acid, other fibrinolytic enzyme inhibitors	None, effect persists throughout lifespan of affected platelets
Efficacy in					
Venous thrombosis	+	+	++	++	?
Pulmonary embolism	+	+	+	++	?
Arterial thrombosis	?	?	?	++	?

\*0.15 M NaCl or 5% dextrose in water

†More detailed laboratory control may be desirable in many cases

‡Evidence is preliminary; controlled trials on large numbers of patients are not as yet available

in the course of acute pulmonary embolism,<sup>181</sup> eg, 10,000 to 15,000 U intravenously every four hours.<sup>69</sup> For prophylaxis, much smaller doses have proved effective, eg, 5000 U subcutaneously every eight hours.<sup>69</sup> The subcutaneous route of administration and various repository forms of heparin<sup>115</sup> may be suitable for prophylaxis, but are not recommended for the treatment of patients with major thrombosis or pulmonary embolism. The intramuscular route of administration is not recommended because of the frequency of complicating hematomas.<sup>129</sup> The dose of heparin should be reduced in patients with thrombocytopenia.<sup>83</sup> Details will be found elsewhere concerning the use of heparin in extracorporeal circulatory devices and in hemodialysis.<sup>83,165</sup> The utility of heparin for *in vitro* blood preservation is discussed on page 8.

### Laboratory Control

There is no entirely suitable method for monitoring the *in vivo* effects of heparin.<sup>153</sup> For many years the whole blood coagulation time has been used for this purpose. When heparin is administered intravenously on an intermittent schedule, the coagulation time, as determined prior to the next dose, should be kept within one and one-half to three times normal or baseline values. The inaccuracies and limitations of the coagulation time as a screening test for bleeding disorders were discussed on page 1057; these apply equally to its use in controlling heparin therapy. Several other simple coagulation tests are useful in monitoring the effects of heparin. They are, in order of decreasing sensitivity, the plasma thrombin time, the partial thromboplastin time (PTT), and the prothrombin time. The last-named test is relatively insensitive to the action of heparin, but may be valuable as a rough index of heparin effects,<sup>83</sup> eg, in neutralizing heparin following surgical procedures involving extracorporeal circulation.

Many laboratories now employ the *activated PTT* for monitoring of heparin therapy.<sup>37,180,181,206</sup> The results of this test cor-

relate closely with the coagulation time at low heparin concentrations. However, the PTT is virtually infinite at high heparin concentrations, a fact which limits its usefulness in monitoring peak levels of heparin.<sup>119</sup> Modifications of the PTT that employ diluted plasma,<sup>119</sup> and simplified methods for the direct assay of heparin concentrations in plasma,<sup>204a</sup> also have been proposed for the control of heparin therapy.

It is probable that the value of frequent monitoring of the effects of heparin, in terms of preventing hemorrhagic complications,<sup>11,181</sup> has been overemphasized. In one study,<sup>153</sup> none of several monitoring techniques, including specific heparin assays, was correlated with the development of bleeding complications in patients receiving this drug. The frequency of bleeding complications appears to be little, if at all, greater among patients in whom no monitoring techniques are employed,<sup>11</sup> or in those receiving high as distinguished from low doses of the drug.

It is well known, however, that heparin requirements vary greatly,<sup>118</sup> and may change during the course of an illness. Large doses may be required in the presence of an active thrombotic lesion; in some patients with massive thromboembolic lesions, marked "heparin resistance" has been noted.<sup>153</sup> Heparin requirements also may diminish rapidly as the thrombotic lesions resolve. It is probable that a daily estimate of the anticoagulant effects of heparin, as provided by the PTT, for example, should be routinely obtained to prevent a progressive build-up of the *in vivo* concentrations of this drug, and also to make certain that the patient is receiving a sufficient amount.<sup>151</sup>

### Complications

Urticaria, fever, transient thrombocytopenia, and other minor adverse effects<sup>62,74,164</sup> have been described in association with heparin administration, but the major complication attending the use of this drug is hemorrhage. The most common hemorrhagic manifestations are ecchymoses at venipuncture sites, and exacerbation of latent hemor-

rhagic lesions, eg, duodenal ulcers. More serious hemorrhagic manifestations have been described,<sup>84</sup> particularly if aspirin or related drugs have been administered together with heparin.<sup>184</sup> They presumably are the result of the combined effects of heparin and impaired platelet function. A similar phenomenon may explain the high incidence of hemorrhagic complications in association with heparin therapy which has been reported in patients with thrombocytopenia<sup>83</sup> and uremia.

Postoperative and post-traumatic hemorrhage may be serious in patients receiving heparin. Intramuscular injections may produce large hematomas, and when given in the thigh or buttocks may produce retroperitoneal hematomas.<sup>129</sup> The incidence of bleeding complications associated with heparin is unaccountably high in postmenopausal women.<sup>104</sup>

In the treatment of bleeding that may complicate heparin therapy, discontinuing the use of the drug is generally all that is required because of the rapidity with which it is normally degraded. If rapid reversal of the effects of heparin is required, *protamine* is an effective and specific antidote. This agent is a strongly cationic protein, which neutralizes the acidic charge on the heparin molecule.<sup>115</sup> Methods for calculating the required dose have been described,<sup>83,168</sup> but unless the heparin level is very high, a single dose of 50 mg of protamine sulfate given intravenously at a slow rate usually is sufficient.<sup>83,168</sup> Protamine neutralization tests<sup>49,83,168</sup> are very time-consuming, but provide a means of determining the exact amount of protamine required. Such tests are seldom required, and empiric neutralization of heparin has proved safe and effective even following major operations on the heart and lungs. Protamine in great excess may act as an anticoagulant, but this action very rarely presents a significant problem.

*Heparin "rebound"* usually refers to the reappearance of heparin in the circulation, following its apparent neutralization by protamine. This phenomenon usually is seen following the use of extracorporeal cir-

culatory devices, and is poorly understood.<sup>68,73a,168</sup>

### Coumarin and Indanedione Anticoagulants

All of the numerous synthetic and natural compounds that antagonize the biosynthesis of the vitamin K-dependent coagulation factors are derivatives of two basic chemical structures, namely, the 4-hydroxy coumarins and the 1,3-indanediones<sup>115</sup> (Table 39-1). The anticoagulant action of all of these compounds is the same; they differ in terms of pharmacologic features such as the rate and extent of their absorption, or the onset and duration of their action. They are absorbed from the gut, bound almost entirely to albumin and are carried in the plasma in this form. They are hydroxylated by the hepatic cells at a constant rate, and are excreted in the hydroxylated form in the urine.<sup>115</sup> The rate of biotransformation varies greatly from individual to individual.<sup>147</sup> The coumarin-like drugs cross the placenta, an attribute of clinical significance<sup>90</sup> (page 1203).

### Mechanism of Action

The coumarin and indanedione drugs do not act in the circulation but, rather, in the liver, where they impair the synthesis of the four vitamin K-dependent coagulation factors, ie, prothrombin, factors VII, IX, and X.<sup>152</sup> The biochemical mode of action of the coumarins is poorly understood. It has been suggested that they may act on two different receptors,<sup>115</sup> upon processes that metabolize vitamin K<sup>13,43,123</sup> or those that transport this vitamin across the cell membrane.<sup>50,115</sup> Although many of these drugs bear a structural resemblance to vitamin K, they do not act by simple metabolite-antimetabolite competition.<sup>3,115</sup> They lead to the production of qualitatively abnormal congeners of the four coagulation factors,<sup>40a</sup> as discussed on page 414, and there is evidence that, as in vitamin K deficiency, they impair the final synthetic step that transforms a common precursor into the various factors (Fig. 10-2, page 415).

There is a considerable lag between peak plasma levels of the coumarin-like drugs and the maximal prolongation of the prothrombin time. This represents the time required for the coagulation factors in the circulation to disappear. The plasma levels of the four factors fall in a sequence consistent with their *in vivo* survival times (Chapter 10), namely, factor VII the most rapidly, followed by factors IX, X, and prothrombin in that order.<sup>110</sup> The clinical effectiveness of the coumarin anticoagulants is mainly the result of reduction of the levels of factors IX and X.<sup>41</sup>

### Factors That Modify the Action of Coumarin Anticoagulants

Many commonly used drugs (Table 39-1) interact with the coumarin anticoagulants. They may act on any of the various mechanisms involved in the absorption, transport, biotransformation, and excretion of these anticoagulants.<sup>43,112,116</sup> Those of one group, eg, barbiturates, antagonize the action of the coumarins.<sup>112</sup> They produce coumarin "resistance," and may lead to overdosage when they are withdrawn. Other drugs, such as the salicylates and phenylbutazone, potentiate the action of the coumarins. In very large doses, salicylates have a coumarin-like effect, but alone rarely produce bleeding. Broad-spectrum antibiotics and nonabsorbable sulfonamides antagonize vitamin K synthesis by gut bacteria, but there is little evidence that this alone is ever of practical significance in facilitating the action of the coumarins.<sup>112</sup> Coumarins may greatly potentiate the actions of dilantin, tolbutamide, and chlorpropanide, but the effects of these drugs in potentiating the action of the coumarins appear to be minor and of uncertain clinical significance.<sup>112</sup> *In vitro* experiments and studies in animals<sup>112</sup> suggest that a large number of other drugs may interact with the coumarins.

The physician should be well aware of the many drugs that can interact with the coumarin anticoagulants. Such interactions are a common cause of erratic and inadequate anticoagulation or overdosage attended with bleeding, or both.

Many other physiologic factors and pathologic processes modify the effects of coumarin drugs.<sup>3,115</sup> Especially important among these are the functional efficiency of the liver<sup>30</sup> and the dietary intake of vitamin K.<sup>188</sup> Infants are unusually sensitive to the action of coumarin-like drugs. Any of the factors that increase the levels of the vitamin K-dependent coagulation factors (page 421) may antagonize the coumarin anticoagulants. In two families virtually absolute resistance to coumarin-like drugs was inherited as an autosomal dominant trait.<sup>145</sup>

### Regimens

Because of a relatively high incidence of adverse reactions, the indanediones have been largely supplanted by various coumarin derivatives, among which warfarin (Coumadin) and bishydroxycoumarin (Dicumarol) are by far the most widely used (Table 39-1). Therapeutic regimens for the many other oral anticoagulants are described elsewhere.<sup>49,50,113</sup>

Following the administration of warfarin or bishydroxycoumarin, the prothrombin time is maximally prolonged in from 36 to 72 hours.<sup>115</sup> However, the antithrombotic action of both of these drugs apparently is not maximal until the plasma levels of factors IX and X are significantly depressed. This may require several days. Consequently, the administration of 10 to 15 mg of warfarin daily until the prothrombin time is within the therapeutic range may be therapeutically as effective and less dangerous<sup>43</sup> than traditional regimens which employ larger initial loading doses.<sup>43,116</sup>

### Laboratory Control

The plasma prothrombin time is sensitive to three of the four vitamin K-dependent coagulation factors. Consequently this test, or a modification thereof, is the preferred method of monitoring the effects of coumarin-like drugs in the laboratory.

A large number of modified thrombo-



plastins, and technical modifications of Quick's original one-stage prothrombin time (page 1056), have been advocated by various authorities. The advantages claimed for these techniques include greater overall sensitivity and precision,<sup>49</sup> and greater or lesser sensitivity to the various coagulation factors. Modifications that make the test less sensitive to the levels of factor V (P and P, Thrombotest) may offer some advantage in the determination of the prothrombin time of mailed or stored specimens. The prothrombin time may be performed on capillary blood with Thrombotest.<sup>49</sup> With these exceptions, however, these modifications probably offer no advantages over the standard one-stage prothrombin time, when it is expertly performed with a well-standardized thromboplastin<sup>116</sup> on a carefully collected blood specimen. In patients receiving coumarin and indanedione drugs, the "therapeutic range" for the prothrombin time, as determined with the usual techniques, is from 1.6 to 2.5 times<sup>49,50</sup> control values.

When it is desired to switch from heparin to one of the coumarin anticoagulants, the two drugs must be given concomitantly for a time, usually for three to five days. During this period, the coagulation time is still a valid indicator of heparin effect, because it is little affected by coumarin-like drugs. However, the prothrombin time is meaningless as an indicator of coumarin effect, because it is prolonged by heparin. The PTT is affected by both coumarin and heparin.

### Complications

Bleeding due to the coumarin drugs is a common complication. Virtually all hemorrhagic manifestations have been encountered, including some serious ones,<sup>49,50,117</sup> eg, bleeding into the central nervous system. Hematuria and gastrointestinal bleeding occur frequently, but are found to originate from localized organic lesions with surprising frequency. Gastrointestinal bleeding from peptic ulcers is one of the most common causes of fatal hemorrhage. As with heparin, the assumption that bleeding can always be

avoided by careful regulation of the dosage of the coumarin drugs is not entirely justified, since, in some patients, bleeding develops when the prothrombin time is within the therapeutic range.

Bleeding usually is minimal following surgical procedures or injuries in patients receiving the coumarin drugs. It is probable that use of these drugs need not be discontinued when common surgical procedures are performed.<sup>166</sup> Aspirin is contraindicated in patients receiving coumarin-like drugs, since inhibition of platelet function may provoke hemorrhage.

Bleeding due to the surreptitious self-administration of coumarin drugs is not uncommon.<sup>4,23</sup> This problem (the *dicoumarol "eaters" or malingers*) is most common in women, and in medical or paramedical personnel. Drug ingestion usually is denied, but puzzling and fluctuating laboratory abnormalities should arouse suspicion. Significant psychiatric abnormalities are present in some patients, but in others protracted self-treatment of resolved or imagined thromboembolic disorders is responsible. The parenteral administration of large doses of vitamin K and the chemical determination of plasma coumarin levels<sup>9</sup> may be required for diagnosis.

The administration of vitamin K usually is advisable in the treatment of hemorrhagic complications in patients who are receiving the coumarin drugs, because of the relatively long duration of action of these agents. The response to vitamin K is more variable than is commonly realized.<sup>205</sup> The administration of 25 mg of vitamin K<sub>1</sub> usually restores the prothrombin time to normal within 24 hours. Larger doses (50 mg) may be required to counteract the effects of longer-acting drugs; in severe toxicity, two to three doses of vitamin K<sub>1</sub> may be required. The absence of a response to vitamin K suggests a complicating process, eg, liver disease, diffuse intravascular coagulation (Chapter 38).

*Skin necrosis* is a rare but dramatic complication of coumarin therapy. These lesions apparently represent a unique form of vascular purpura, and usually develop during the first week of treatment. They are most com-

mon in women, and usually involve the legs, breasts, and external genitalia. They may be very painful,<sup>189</sup> but seldom produce serious sequelae. A symmetrical, purplish, painful discoloration of the toes also may develop as a consequence of coumarin therapy.<sup>59</sup> The pathophysiology of this manifestation is obscure.

## Miscellaneous Antithrombotic Agents

### Therapeutic Defibrination

In 1963, Reid and associates<sup>162</sup> observed that envenomation by the Malayan pit viper (*Ancistrodon rhodostoma*) produces total afibrinogenemia with incoagulable blood, in the absence of significant hemorrhage or vascular occlusion. Investigation of this remarkable phenomenon has led to the development of a unique form of antithrombotic therapy which employs an enzyme purified from the venom of this snake. This agent (*ancrod*, *Arvin*) is capable of rapidly removing fibrinogen from the circulating blood, without producing significant alterations in other coagulation factors or the platelets (Table 39-1).<sup>56</sup> It is a potentially useful therapeutic agent, but at present remains in the investigational stage.<sup>187</sup>

### Mechanism of Action

As purified from the crude venom, ancrod is a thrombin-like proteolytic enzyme which has a molecular weight of approximately 30,000 and contains 20% carbohydrate.<sup>56</sup> It converts fibrinogen into an abnormal soluble fibrin polymer.<sup>10,74</sup> Much of this fibrin remains in solution, but "microclots" also are formed<sup>177</sup>; both forms are removed from the circulation by the reticuloendothelial system<sup>55</sup> and as the result of rapid fibrinolysis.<sup>55,58</sup> Crude venom contains a second enzyme capable of lysing circulating fibrinogen,<sup>160</sup> but this is not present in ancrod.

The proteolytic action of ancrod on fibrino-

gen results in the removal of the fibrinopeptides A and several abnormal basic peptides,<sup>156</sup> but peptide B is not removed.<sup>55,93</sup> It does not activate factor XIII, which may explain the susceptibility to fibrinolysis of the fibrin produced by ancrod.<sup>156</sup>

The active principle in ancrod is rapidly degraded in vivo, probably by the reticuloendothelial system<sup>161</sup>; it also is inactivated by alpha-2 macroglobulin and antithrombin III (page 431).<sup>154</sup> A specific antivenom that rapidly reverses the effects of this agent in vivo has been developed.<sup>187</sup>

### Efficacy

Studies of experimental thrombosis in animals have suggested that ancrod is capable of preventing venous thrombosis<sup>122</sup> and favorably affects pulmonary embolism.<sup>143</sup> Studies in animals suggesting that this enzyme may accelerate the removal of preformed thrombi<sup>122</sup> could not be confirmed in human subjects.<sup>109,159</sup> In one animal model, ancrod treatment significantly reduced the incidence of arterial thrombosis.<sup>187</sup>

Preliminary clinical trials in man have demonstrated encouraging therapeutic responses in venous thrombosis,<sup>15,159,174</sup> retinal vein thrombosis,<sup>22</sup> and long-standing priapism.<sup>14</sup> There is little evidence, however, that ancrod is any more effective than heparin in the treatment of most of these disorders.<sup>109</sup> Carefully controlled studies in large groups of patients have yet to be reported.

Therapeutic defibrination with ancrod is remarkably free of adverse effects.<sup>187</sup> The only significant side effect encountered in experimental animals was disseminated microthrombosis, a complication that developed only when the enzyme was administered too rapidly.<sup>7,121</sup> In man, spontaneous hemorrhage is very rare, but postoperative bleeding has been described.<sup>174</sup> Microangiopathic hemolytic anemia, presumably due to the effects of intravascular fibrin deposits,<sup>174</sup> has been noted in an occasional patient, as has defective wound healing.<sup>94,174</sup> Ancrod is antigenic,<sup>15,155,174</sup> a drawback that may somewhat limit its overall usefulness.

## Thrombolytic Therapy

In thrombolytic therapy, the endogenous fibrinolytic enzyme system is activated by the administration of plasminogen activators, with the object of inducing or accelerating the lysis of a preformed thrombus<sup>175</sup> (Table 39-1). Thrombolytic therapy is thus directed at the thrombus *per se*, rather than at the causes of thrombosis.<sup>61</sup>

### Mechanism of Action

The only agents presently available for thrombolytic therapy are streptokinase and urokinase. Synthetic agents<sup>61</sup> and enzymes derived from plants<sup>90</sup> are presently in the developmental stage. Streptokinase is antigenic,<sup>65</sup> and its administration produces fever and other adverse effects. Urokinase of high purity avoids these problems, but is expensive and in very short supply, even for investigative trials.

The infusion of urokinase and streptokinase in full therapeutic doses results in the prompt appearance of plasminogen activators in the circulating blood, and the presence of variable amounts of free plasmin (Fig. 10-7, page 433). This leads to fibrinolysis, and to fibrinogenolysis of variable degree. The mechanisms by which this occurs, and the many laboratory abnormalities that may result, are described in detail in Chapters 10 (page 432) and 38 (page 1244). A considerable excess of both streptokinase and urokinase must be administered to overcome plasma inhibitors; the "thrombolytic state" can then be maintained by the administration of smaller maintenance doses of either enzyme.<sup>124</sup> The dosage of both agents must be individualized, since the responses of individual patients vary greatly. With most regimens, plasminogen depletion occurs rapidly.

Antifibrinolytic agents such as EACA and tranexamic acid are potent and effective antidotes to the effects of thrombolytic agents.

### Efficacy

The evidence that thrombolytic therapy is capable of lysing thrombi in animals without undue hazard of hemorrhage is now convincing.<sup>124,175</sup> This mode of therapy has been thoroughly evaluated in only a small number of patients, although there are many reports of uncontrolled trials, and many others in which benefit was claimed even though only homeopathic doses of thrombolytic agents were administered. The most clear-cut evidence for the clinical effectiveness of thrombolytic therapy has been obtained in patients with peripheral arterial thrombosis.<sup>99,114,124</sup> In patients with thrombosis of leg veins<sup>184</sup> and in those with pulmonary embolism,<sup>172,184</sup> thrombolytic therapy accelerated the recanalization of affected vessels, an effect not observed in heparin-treated controls. It is uncertain, however, whether the overall therapeutic effectiveness of streptokinase and urokinase was significantly greater than that of heparin.<sup>102,127,183,184</sup> In myocardial infarction, a slight but significant reduction in mortality was produced by thrombolytic therapy.<sup>5,191</sup>

The efficacy of thrombolytic therapy is diminished if it is not given early, since older thrombi are resistant to lysis.<sup>67</sup> Thrombi that form after plasminogen has been depleted also are resistant to further lysis, and for this reason anticoagulants usually are administered following the cessation of thrombolytic therapy.<sup>175</sup>

The results of preliminary studies of thrombolytic therapy have been encouraging; the enzymes, streptokinase and urokinase, are among the few presently available therapeutic agents that offer promise in the treatment of arterial thrombosis.<sup>57</sup> Additional large, carefully controlled clinical trials of thrombolytic agents are certainly indicated.<sup>175</sup>

### Drugs That Impair Platelet Function

Theoretically, agents that impair platelet function might be therapeutically effective in

the treatment for arterial thrombosis (Table 39-1). An intensive study of the antithrombotic properties of the numerous compounds, discussed in Chapter 35, that inhibit platelet functions in vitro is presently underway.<sup>195</sup>

*Dipyridamole*, a drug initially marketed as a coronary vasodilator, is a potent inhibitor of the platelet release reaction (page 394). It is effective in inhibiting experimental arterial thrombosis in rabbits<sup>51</sup> and rats,<sup>46</sup> and markedly reduced the incidence of thromboembolic complications in patients with prosthetic heart valves.<sup>51,182</sup> It has proved ineffective as an antithrombotic agent under other circumstances.<sup>2,28,170,195</sup> Analogs of this compound are currently under intensive study.<sup>53</sup>

*Aspirin*, in very small doses, is a potent inhibitor of the platelet release reaction. Studies in animals suggest that this drug has a significant antithrombotic effect,<sup>47,195</sup> and preliminary trials suggest that it may be useful in the prevention of ischemic heart disease in man.<sup>20a,53a</sup> Other therapeutic trials in man have been disappointing.<sup>142,144</sup> Aspirin apparently is not therapeutically beneficial in preventing venous thrombosis in patients with hip fractures<sup>201</sup> or in the treatment of patients with leg vein thrombosis.<sup>104</sup> Large cooperative studies of the effects of this drug in arterial thrombosis, such as that involved in transient ischemic attacks and ischemic heart disease, are underway.

*Dextran* of low molecular weight has been shown to inhibit platelet aggregation and the release reaction in vitro. It also may have an "antiadhesive" effect on vascular surfaces, and a weak heparin-like effect when given in high concentrations.<sup>49</sup> This polymer appeared to prevent thrombosis of the leg veins in some clinical trials,<sup>85</sup> but not in others.<sup>27,170,181</sup> Evidence that it is effective in the prevention or treatment of thromboembolic disorders is less convincing than is the case with either heparin or the coumarins. Despite this fact, dextran has gained surprisingly widespread acceptance in the treatment of patients with thrombosis.

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# Part V

## Disorders Characterized By Adenopathy, Splenomegaly, and/or Abnormalities of Leukocytes or Immunoglobulins

In the following chapters, disorders which primarily involve the immune system (lymphocytes, lymph nodes, plasma cells, and immunoglobulins) and the mobile phagocytic system (neutrophils, eosinophils, and monocytes) will be considered. Conditions leading to detectable abnormalities of these systems may be self-limited and benign or they may be serious. As with Disorders of the Red Cells (Part III) and Disorders of Platelets (Part IV), the cellular disorders to be discussed here can be classified as abnormalities of cellular excess, cellular deficiency, or intrinsic cellular abnormality. Cellular excess or deficiency is detected by finding enlarged lymph nodes or splenomegaly on physical examination or increased or decreased concentrations of leukocytes in the blood or bone marrow. Intrinsic cellular abnormality may be accompanied by detectable morphologic changes or its diagnosis may require tests of cell function or determination of biochemical composition. As in the other cellular systems (Parts III and IV), discussion of the specific types of abnormality and disease affecting the immunocytic and phagocytic systems will follow consideration of the overall diagnostic approach to the patient with these conditions.

## SECTION 1: *Approach to Disorders of the Phagocytic and/or Lymphatic Systems*

This section presents an *orderly* approach to diagnosis of disorders characterized by lymphadenopathy, splenomegaly, and/or abnormalities of leukocytes or immunoglobulins. These disorders may take the form of (1) reactions to other diseases (changes in blood leukocytes and/or enlarged lymphoid tissue), (2) neoplastic diseases (leukemias, lymphomas, and immunoglobulin-producing tumors), and (3) idiopathic or inherited diseases due to defective phagocytosis or antibody production.



### *Diagnostic Steps in the Evaluation of the Patient with Abnormalities of Leukocytes or Immunoglobulins, or Lymphadenopathy, Splenomegaly, Fever of Unknown Origin, or Recurrent Infection*

Examination of the Blood  
Bone Marrow Examination  
Lymph Node Examination  
Examination of the Spleen  
Fever of Unknown Origin  
Recurrent Infections

THE patient may have no symptoms referable to a disorder of the phagocytic or immune systems or there may be signs and symptoms suggesting infection. Other common presenting complaints, especially in patients with neoplastic disease, include fever without evident infection, fatigue, enlarged lymph nodes or other masses, abnormal bleeding, weight loss, pain in bones and joints, and

generalized itching. Suspicion that one is dealing with such a disorder may be raised during the course of routine examination, or during evaluation of unrelated disorders, by the detection of lymphadenopathy, splenomegaly, or hilar or mediastinal masses. In other instances, blood examination discloses leukocyte abnormalities such as leukopenia, leukocytosis or abnormal cells, anemia and/or platelet abnormalities, or altered levels of immunoglobulins.

A detailed history should be obtained and a thorough physical examination made. Chest x ray, urinalysis, blood examination (including study of red cells, white cells, platelets, and the blood smear), marrow examination, lymph node biopsy, liver biopsy, splenec-

tomy, and paper electrophoresis and immunoelectrophoresis of serum or urinary proteins may be necessary. In addition, evaluation of delayed hypersensitivity and tests of neutrophil function may be required. Obviously not all or even most of these tests and surgical procedures are indicated in each patient who has the symptoms described above. Rather, a logical sequence of studies is undertaken in each patient, tailored to the particular findings. A diagrammatic summary of diagnostic sequences is shown in Figure 40-1.

## Examination of the Blood

The blood may be entirely normal in some patients with lymphoma, myeloma, immune deficiency, neutrophil dysfunction, or a variety of other syndromes associated with lymphadenopathy or splenomegaly. Anemia, when present, usually is normochromic and normocytic. However, some patients with "preleukemic" myeloblastic leukemia (Chapter 47) or with multiple myeloma (Chapter 52) have anemia but no other symptoms. Anemia of unknown origin should lead to marrow examination as well as serum and urine protein electrophoresis.

Thrombocytopenia and/or thrombocytosis with no other signs, symptoms, or blood findings suggesting the diseases under consideration is extremely rare, having been described in only a few patients with "preleukemic" myeloblastic leukemia (Chapter 47). However, thrombocytopenia commonly accompanies other blood changes in diseases such as the acute leukemias (Chapter 47) as does thrombocytosis in chronic myelocytic leukemia (Chapter 48).

Changes in blood leukocytes, if present, may, in themselves, provide the diagnosis, as in the leukemias, may strongly suggest the correct diagnosis, or may be nonspecific but still indicative of certain specific conditions. Certain rare diseases associated with frequent infection can be recognized by morphologic changes in leukocytes (eg, Chediak-Higashi syndrome,<sup>5</sup> Chapter 42).

Leukocyte changes in the blood of patients with acute leukemia (Chapter 47) provide the

diagnosis in more than half such patients and strongly suggest it in virtually all. In approximately 60% the smear confirms the diagnosis by virtue of the presence of a large proportion of immature cells, whether or not there is an increased leukocyte count.<sup>6</sup> Blasts are easily demonstrable in blood smears of more than 90% of patients and, even in patients in whom few blasts are present in the blood smear, anemia, thrombocytopenia, neutropenia, or combinations of these changes suggest acute leukemia. In almost all such patients, bone-marrow aspiration proves diagnostic.

More than  $10.0 \times 10^9$  small lymphocytes/l of blood is usually indicative of chronic lymphocytic leukemia, especially if the patient is middle-aged or elderly, since more than a slight elevation in the number of cells is unusual in other diseases (Chapters 41 and 49). Characteristic signs and symptoms of chronic lymphocytic leukemia may be absent at a time when a diagnosis can be made from examination of a blood smear (Chapter 49).

The presence of chronic myelocytic leukemia (Chapter 48) may be strongly considered on the basis of a blood smear. However, the diagnosis depends upon other findings in addition to neutrophilia. For instance, in the presence of *all* of the following a firm diagnosis can be reached without other considerations: (1) symptoms limited to fatigue, bone pain, left upper-quadrant mass or fullness; (2) sternal tenderness and splenomegaly revealed by physical examination, without other abnormalities excepting pallor or, rarely, tenderness in bones other than the sternum; (3) more than  $100.0 \times 10^9$  leukocytes/l of blood with abundant mature neutrophils and myelocytes with lesser numbers of blasts and promyelocytes and with increased basophils and eosinophils; (4) thrombocytosis and normocytic, normochromic red cells with or without anemia. With any slightly atypical features, a "leukemoid reaction" (Chapter 41), usually due to infection or carcinoma, or disorders such as idiopathic myelofibrosis (Chapter 57), must be considered. However, if cytogenetic examination of the marrow reveals the Philadelphia chromo-

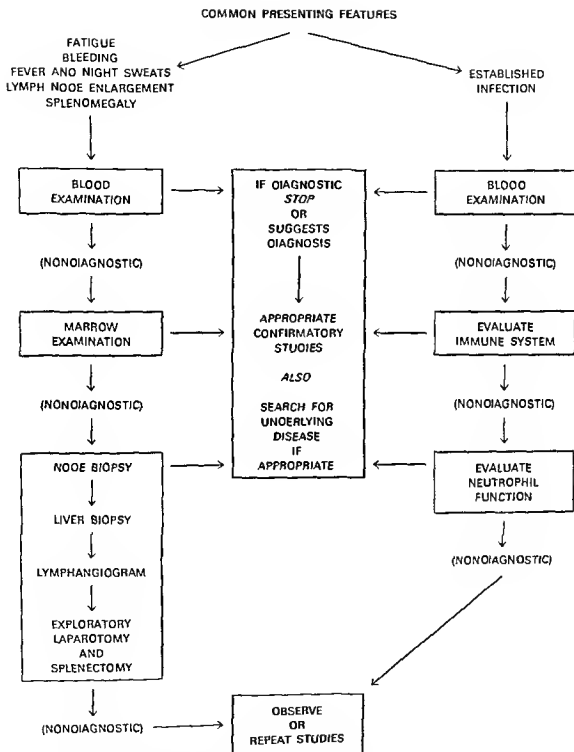


Fig 40-1 Diagnostic steps in diseases of the phagocytic and immune systems

some (Chapter 46), a reasonably firm diagnosis of chronic myelocytic leukemia can be made.

Patients with multiple myeloma may have a few plasma cells in the blood and, occasionally, large numbers (plasma cell leukemia, Chapter 52). Such patients almost invariably have symptoms and signs of the disease. A few cells resembling plasma cells may be observed in blood smears of patients with infectious mononucleosis or other viral infections. Plasma cells also are seen in the blood of persons recovering from bacterial infections or having allergic reactions, especially serum sickness.

The presence of other rare diseases such as systemic mastocytosis (Chapter 47) can be strongly suspected when large numbers of mast cells are seen on blood smears.

Blood leukocyte changes requiring diagnostic consideration which are secondary to a variety of diseases (Chapters 41 and 43) usually consist of neutrophilia, eosinophilia, monocytosis, or normal- or abnormal-appearing lymphocytes. If the leukocyte change is appropriate to the clinical situation, eg, pneumococcal pneumonia with  $200 \times 10^9$  segmented and band form neutrophils/l, the obvious disease should be treated and the blood reevaluated when this has been accomplished.

The causes of unexplained neutrophilia are considered in Chapter 41 as are those for persistent eosinophilia and monocytosis.

Abnormal-appearing lymphocytes such as those seen in infectious mononucleosis must be distinguished from lymphoblasts. This distinction is easily made by the experienced morphologist<sup>12</sup> (Chapter 43). The presence of such cells even in the absence of signs and symptoms characteristic of the disease should raise the question of infectious mononucleosis and appropriate heterophil antibody tests should be carried out (Chapter 43). Such cells also are observed in a variety of viral and protozoal infections such as measles, infectious hepatitis, and toxoplasmosis. If the reaction to the heterophil test is negative, serologic studies for the detection of such diseases should be performed. If no diagnosis is es-

tablished, patience and further observation are advisable. Lymph node biopsy and marrow examination may or may not reveal similar abnormal cells, but they will provide little diagnostic information beyond that obtained from the blood smear.

Neutropenia may provide an explanation for the presence of infection (page 1263). This finding demands consideration of its various causes (Chapter 41). Lymphopenia is produced by a wide variety of causes but is usually a transient phenomenon (Chapter 41). Persistent lymphopenia suggests a defect in cellular immunity such as that present in Hodgkin's disease (Chapter 50).

## Bone Marrow Examination

If a diagnosis is not established by examining the blood, bone marrow examination may prove helpful. If lymphoma or any granulomatous or carcinomatous process or myelofibrosis is under consideration, needle biopsy should be performed in addition to aspiration (Chapter 2). As noted in the previous section, confirmation of a suspected diagnosis of acute leukemia or of myeloma is obtained by means of marrow aspiration. Except for cytogenetic studies, marrow examination provides no more diagnostic information than does blood examination in the chronic leukemias.

A diagnosis of lymphoma may sometimes be established from examination of the bone marrow, especially if biopsy is performed (Chapter 51). Evidence of disease in the marrow was found in 30% of patients with lymphocytic lymphoma, 26% of patients with giant follicle lymphoma, and 19% of patients with reticulum cell (histiocytic) lymphoma.<sup>29</sup> However, marrow involvement was found in fewer than 10% of patients with Hodgkin's disease.<sup>8</sup> Biopsy of bone marrow also is useful in evaluating the extent of lymphomatous disease (staging, Chapters 50 and 51) once the diagnosis has been confirmed. Thus, marrow examination can be justified in any patient suspected of having a lymphoma and in a minority of the patients this examination will yield a definitive diagnosis.

If patients complain of bone pain, the bone marrow should always be examined whether or not bone lesions have been demonstrated radiographically. However, multiple myeloma is occasionally quite focal in nature and in such instances plasmacytosis may not be detected in the area of marrow which is examined. Marrow aspiration or biopsy of a site suggested by radiography should then be considered. Serum protein electrophoresis and a test for Bence Jones protein in the urine should also be routinely performed in such patients. Only some 2% of patients with myeloma have neither a "spike" on electrophoresis of serum proteins nor Bence Jones protein in the urine (Chapter 52).<sup>28</sup>

The indications for marrow examination in the evaluation of the patient with fever or infection are considered later in this chapter (page 1263).

## Lymph Node Examination

This section is concerned with the evaluation of the patient with palpable or visible enlargement of cervical, supraclavicular, axillary, inguinal, mediastinal, or hilar lymph nodes or tonsils. If the blood and marrow (when indicated, as discussed in the previous sections) fail to provide a diagnosis, biopsy of enlarged lymphoid tissue should be considered. The presence of a variety of signs and symptoms may modify and shortcut the diagnostic evaluation.

How large must lymphoid tissue be before it is considered abnormal? Exact limits cannot be set. The answer varies with the age of the patient, his occupation, and the location of the lymph nodes. Careful physical examination by an experienced physician reveals palpable small lymph nodes and visible tonsillar tissue in almost all patients.

The most common cause of lymphoid enlargement is cellular proliferation (antibody production) in response to antigenic stimulation. The frequency of trychophyton infection of toes and trauma to feet and legs is reflected in the frequency of palpable inguinal nodes (usually less than 1 cm in diameter in most patients). If the patient's occupation or

hobby leads to frequent trauma of hands and arms, then small palpable epitrochlear and axillary nodes are expected. Thus, the assessment of the lymphoid system in a laborer differs somewhat from that of an educator (unless the latter's hobby is gardening and hunting).

Children and adolescents commonly have more palpable nodes than do adults. This is presumed to reflect their more frequent exposure to antigens new to their experience. Thus, significant generalized adenopathy has a more liberal definition in the young than in adults. This is particularly true of tonsils. Rather prominently visible tonsils, protruding into the oropharynx, are expected in preschool children but are uncommon in adults.

Thus, clinical judgment based on experience with many patients is necessary in the diagnosis of "abnormally" enlarged lymphoid tissue. If lymphoid tissue is unequivocally enlarged, however, prompt, thorough evaluation is indicated. If doubt exists as to whether there is significant enlargement, the choice between immediate biopsy or periodic observation to discover whether the nodes will enlarge or shrink should be made on the basis of ancillary findings. If there are other signs and/or symptoms suggesting a lymphoma, biopsy of small, questionably enlarged nodes may yield a diagnosis. If it is decided to delay biopsy, then periodic observation should not be allowed to lead to procrastination. Biopsy should be performed after no more than one or two months of observation if the physician is still suspicious that the nodes are abnormal.

The causes of enlargement of lymph nodes are extremely varied. In Table 40-1, the major categories of disease leading to lymphadenopathy are presented. This table is in no sense exhaustive.

Certain physical characteristics of nodes aid in the diagnosis of the cause of lymphadenopathy. Infected nodes usually are tender and the overlying skin often is inflamed. Such nodes may be matted together. In certain types of infection, such as tuberculosis, aspergillosis, or actinomycosis, sinus tract formation is common. Infected nodes may be

Table 40-1. Conditions Leading to Lymph Node Enlargement

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I	Lymphadenopathy due primarily to immune response
A	Infections
1	Pyogenic infections
a	Local enlargement of nodes draining areas of local infection (eg furuncles caused by staphylococci, oral infection)
b	Generalized enlargement from fairly indolent infections (eg <i>Salmonella</i> septicemia, bacterial endocarditis)
2	Viral infections
a	Local enlargement of nodes draining portals of entry of infection (eg cat scratch fever, lymphogranuloma venereum)
b	Generalized lymphadenopathy with systemic infections (eg infectious mononucleosis, measles, infectious hepatitis)
3	Miscellaneous types of organisms
a	Local enlargement of nodes draining the portal of entry of such infections as cryptococcosis, primary chancre of syphilis
b	Generalized adenopathy (eg secondary syphilis, toxoplasmosis)
B	Lymphadenopathy secondary to an immune response to noninfectious agents (eg, serum sickness following injection of foreign protein)
II	Lymphadenopathy due primarily to infection of the node by organisms
A	Pyogenic infection, the classic example is the bubo of <i>Pasteurella pestis</i> , more commonly abscess formation by staphylococcal invasion
B	Granuloma formation. The entrance of tubercle bacilli or fungi such as <i>Histoplasma capsulatum</i> into nodes often results in granuloma formation as well as hypertrophy, identifiable organisms are present within the granuloma in many instances
III	Neoplastic evolution or invasion of nodes
A	Primary neoplastic diseases of nodes
1	Hodgkin's disease and non-Hodgkin's lymphomas (NHL) (Chapters 50 and 51)
2	Lymphoid leukemia: chronic lymphocytic leukemia, acute lymphoblastic leukemia (Chapters 47 and 49)
3	Other neoplastic disease of nodes such as lymphoepithelioma
B	Secondary neoplastic processes occurring in nodes
1	Myeloid leukemias, acute myeloblastic leukemia, chronic myelocytic leukemia (Chapters 47 and 48)
2	Idiopathic myelofibrosis with extramedullary hematopoiesis producing lymph node enlargement (Chapter 57)
3	Metastases from carcinoma producing lymph node enlargement
IV	Diseases of unknown cause leading to lymph node enlargement (usually generalized)
A	Autoimmune diseases (diseases which perhaps result from immunologic recognition of the patient's tissue as foreign tissue)
1	Systemic lupus erythematosus, rheumatoid arthritis, and other "collagen vascular" diseases
B	Reaction to drugs
1	Hydantoins and related chemicals
C	Miscellaneous diseases
1	Granuloma formation as seen with sarcoid or in patients exposed to beryllium
2	Reactive hyperplasia as seen in hyperthyroidism

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fluctuant. Nodes undergoing an immune response to infection also may be tender, but other signs of inflammation usually are absent. Tenderness may be due to other conditions but this is uncommon. Carcinomatous nodes usually are very hard, may be bound to one another and to surrounding tissues; lymphomatous nodes are more often firm, rubbery, discrete, and freely movable.

The location of the nodes may be helpful.

Large, bilateral hilar nodes without other detectable adenopathy are unusual in patients with lymphomas and are more suggestive of sarcoidosis.

Careful questioning of the patient is necessary since trauma or infection distal to the local enlargement may have been forgotten or the patient may fail to mention recent systemic symptoms which could account for generalized enlargement. It is known that

animals, given antigen subcutaneously, produce most of the specific antibody in nodes draining the injection site; intravenous antigen results in widespread antibody production, particularly in the spleen.<sup>26</sup> Considering the variety of diseases associated with lymphadenopathy (Table 40-1) it is apparent that many systems of the body must be assessed and any symptoms, signs, or laboratory abnormalities carefully evaluated. Cervical node metastases from a nasopharyngeal tumor may overshadow the primary tumor which is often small and easily overlooked unless a careful nasopharyngeal examination is made.<sup>21</sup> Relatively asymptomatic infection with parasites of the genus *Toxoplasma* is quite common in many parts of the world and may produce lymphadenopathy.<sup>18</sup> Determination of dye titers to the organism is thus a useful screening procedure for patients with unexplained adenopathy and if the toxoplasmosis is active a rising titer should be found. If evaluation discloses or suggests a self-limited process which reasonably explains enlarged lymph nodes, observation rather than immediate biopsy is justified.

As noted in the preceding sections, in certain situations, as when leukemia or multiple myeloma is suspected, bone marrow biopsy in addition to careful examination of the blood can be justified before performing a lymph node biopsy. Sometimes needle biopsy of the liver may be considered preceding lymph node biopsy, especially if the liver is enlarged.

In general, excisional biopsy is the procedure of choice. However, if there is a strong presumption of metastatic carcinoma or direct infection of the node, needle biopsy or aspiration<sup>2,9</sup> may be considered. The procedure for *lymph node puncture* is simple. The overlying skin is cleansed and sterilized. No anesthesia is necessary. Using a 20-gauge sterile needle and dry syringe the operator grasps the node securely between thumb and index finger and pierces the node. The tissue is aspirated rapidly and the needle is then withdrawn. Smears of the aspirate are made on a clean slide and stained with Wright's stain. Bacterial culture and staining by

Gram's method should also be carried out. A diagnosis of Hodgkin's disease, non-Hodgkin's lymphoma, lymphadenitis, or other conditions causing lymph node enlargement (Table 40-1) may be established by lymph node puncture.<sup>2,9,34</sup> However, excisional biopsy yields much more tissue for diagnostic studies, facilitating multiple histologic section preparation for routine and special stains as well as providing more adequate material for culture. Smears from lymph node puncture allow more exact definition of individual cells than do sections from biopsy, but if imprints are made from the biopsy material this advantage is negated. Histologic diagnosis depends upon changes in the overall architectural pattern of the lymph node as well as upon identification of individual cells, and sections from excisional biopsy specimens are superior to material obtained by needle biopsy of lymph nodes for histologic study. As a consequence, lymph node puncture should be restricted to the very rare patient who refuses to allow performance of excisional biopsy but will allow lymph node puncture.

For excisional biopsy, preference is given to the largest palpable node available. More than one node should be removed, if possible, and irradiated areas should be avoided. As a general rule, biopsy of cervical or supraclavicular nodes is preferable to biopsy of axillary nodes and any of these is preferable to biopsy of inguinal nodes when other considerations are equal. As previously mentioned, inguinal nodes are frequently enlarged from chronic foot infection and when this is the case the architecture often is distorted. Axillary nodes, unless markedly enlarged, may be difficult to find during surgical procedures because of the complex and changed anatomy during exposure of this area. All biopsied nodes should be cultured for various organisms as well as examined in stained sections.

*Imprint preparations* of biopsied lymph nodes also are helpful in the detailed morphologic study of cells. Imprints are made by gently pressing the cut surface of the node on a clean glass slide. This should be done repeatedly as the initial imprint may yield too



thick a cellular layer for accurate morphologic study of individual cells. The slides are stained with Wright or Giemsa stains as for the study of blood smears (Chapter 1).

A mediastinal mass without other physical evidence of disease is found in a few patients with Hodgkin's disease or non-Hodgkin's lymphoma (NHL). Mediastinoscopy may yield a correct diagnosis, allowing one to avoid the more major procedure of thoracotomy.

The results of histologic examination of lymph node biopsy specimens are generally divisible into the following five categories:

1. Hodgkin's disease or NHL. Unless the patient has received medication such as the hydantoin which can produce "lymphomatous" changes that may be reversible when medication is discontinued (Chapter 50),<sup>15,20</sup> the diagnosis is established.

2. Specific infections such as tuberculosis or histoplasmosis

3. Carcinoma.

4. Granulomatous changes without demonstrable infecting organisms. If caseation is present, it is assumed that the lymphadenopathy is a tuberculous or other infectious process; routine culture may disclose tuberculosis or fungal infections. Noncaseous granulomas are encountered in nodes removed from patients with a wide variety of diseases such as sarcoid, beryllium intoxication, Hodgkin's disease, Rocky Mountain spotted fever, and Q fever.

5. "Reactive hyperplasia" or nodes which are normal in pathologic appearance

A histologic appearance of reactive hyperplasia or of nonspecific granulomatous changes does not rule out the possibility of Hodgkin's disease or NHL. Since enlarged nodes from patients known to have Hodgkin's disease may not show evidence of the disease,<sup>16</sup> it is not surprising that the first biopsy may give negative findings in a patient in whom a second biopsy gives positive indication of the condition (Chapter 50). In lymphocytic lymphoma, as discussed in Chapter 51, the infiltration may be very orderly and the lack of distortion of normal nodal architecture leaves one hesitant to make this diagnosis. If more than one node is removed

initially, the likelihood of positive findings is enhanced. In nondiagnostic biopsies the decision to obtain more tissue for further study must be individualized. If there are no other enlarged nodes and there are few or no symptoms, observation may be advisable. Prompt diagnosis and proper therapy are quite important in bacterial and fungal infections and perhaps in Hodgkin's disease (Chapter 50), but are less important for the eventual outcome of the other conditions listed in Table 40-1.

## Examination of The Spleen

Many of the conditions leading to lymphadenopathy (Table 40-1) also produce a palpably enlarged spleen. Consequently, the preceding discussion regarding evaluation of the patient with lymphadenopathy is equally applicable to the patient with splenomegaly. When splenomegaly is associated with systemic infection (acute splenic tumor), the spleen usually is barely palpable, soft to firm, but not hard, and splenomegaly disappears shortly after recovery from the infection. A palpable spleen usually indicates disease, but we agree that a soft spleen tip can be palpated in approximately 1 to 2% of apparently healthy persons.<sup>23</sup> The differential diagnosis of splenomegaly is discussed in detail in Chapter 45.

Splenic puncture (Chapter 45) may be helpful in certain conditions (eg, leishmaniasis, kala-azar<sup>24,33</sup>). As compared to aspiration, needle biopsy increases the chance of a correct diagnosis being reached, but biopsy carries a greater risk than does aspiration.<sup>4</sup> Diagnostic splenectomy is required in some patients, especially those in whom the only palpably enlarged tissue is the spleen. However, before a diagnostic splenectomy is considered, numerous other studies should be made; in most cases these will indicate the diagnosis. For instance, splenic hypertrophy due to increased red cell destruction, such as in hereditary spherocytosis (Chapter 21) and thalassemia (Chapter 26), or that associated with increased platelet destruction, as in idiopathic thrombocytopenic purpura (Chapter 34), should be detected by means of appro-

priate blood and other examinations. Biopsy of enlarged nodes should always be carried out before splenectomy is considered. Liver function tests and needle biopsy of the liver should be done to exclude, among other considerations, the possibility that the splenomegaly is due to hepatic disease producing increased portal pressure. Congestive splenomegaly caused by extrahepatic vascular obstruction can often be detected by a percutaneous splenoportogram. However, if such is the case, an abdominal operation is indicated and the added hazard of the splenoportogram may not be justified.

Indications for splenectomy are discussed in Chapter 45 and elsewhere, in relation to the various diseases associated with splenomegaly.

## Fever of Unknown Origin (FUO)

On occasion, the presenting manifestation of patients with Hodgkin's disease and, more rarely, those with non-Hodgkin's lymphoma may be fever, often accompanied by weight loss and occasionally by generalized pruritus, but without palpably enlarged lymphoid tissue, no mediastinal disease evidenced by chest x ray, and with normal or nondiagnostic blood or bone marrow. In contrast, when fever is the presenting manifestation of acute leukemia, examination of the blood or at least of the bone marrow, should reveal the diagnosis. Thus, the lymphomas must be considered in the workup of a patient with FUO.

In such a patient it may be wise to do a needle biopsy of the liver whether or not hepatomegaly is present. Such a procedure rarely yields a diagnosis of lymphoma unless the liver is enlarged but the possibility of other fever-producing diseases such as visceral or miliary tuberculosis may be ruled out. For the same reason, bone marrow biopsy and culture should be performed.

Fever due to lymphoma with no palpable disease is usually associated with retroperitoneal lymph node enlargement. The most reliable nonsurgical technique for evaluating retroperitoneal lymph nodes is lymphangiography (Chapter 50). However, certain abdominal nodes such as the pararectal, perivesical, omental, and those of the hilus of the spleen and liver are not made visible by radiopaque dye introduced into lymphatics of the feet.<sup>16</sup> If the lymphangiogram discloses enlarged nodes, exploratory laparotomy with node biopsy, open liver biopsy, and perhaps splenectomy are in order. If the lymphangiogram demonstrates no abnormality, exploratory laparotomy may still occasionally reveal lymphoma. In general, if there are no signs, symptoms, or laboratory or radiologic findings suggesting intra-abdominal disease, diagnosis of FUO by exploratory laparotomy is successful in only a very small percentage of patients.<sup>3</sup>

## Recurrent Infections

Although severe infection may develop for no apparent reason in otherwise healthy individuals, recurrent infection should raise the suspicion of defects in cellular or humoral defense mechanisms.

The blood of any patient with infection should be examined carefully. In the presence of pyogenic bacterial infection severe enough to produce fever, neutrophilia is anticipated. If neutrophilia is absent, or indeed if the patient is neutropenic (less than  $1.8 \times 10^9$  neutrophils/l), a neutrophil deficit should be suspected as the cause of the infection (Chapter 42). It must be remembered, however, that overwhelming infections such as pneumococcal pneumonia involving multiple lobes of the lung may produce neutropenia in the absence of leukocytic disease through increased neutrophil utilization in the infection<sup>22</sup> (Chapter 41). In addition, neutrophilia is commonly absent in certain bacterial infections such as brucellosis and typhoid fever.<sup>27</sup> However, in most instances of pyogenic bacterial infections, neutropenia is indicative of overwhelming sepsis or of an underlying factor in the development of the infection.

In repeated infections, evaluation of the immune system and the functional capacity of the neutrophils is in order even if the quantitative neutrophil response to infection is appropriate. Immunologic deficiency is a

more common cause of increased susceptibility to infection than are functional defects of the neutrophil response. Hypogammaglobulinemia is usually accompanied by pyogenic bacterial infection, whereas defects in cellular immunity lead to viral infections such as herpes zoster, or fungal infections such as candidiasis or tuberculosis. Nevertheless, while the type of recurrent infection often points towards a particular defect, both the antibody-producing system and the cellular immune system should be investigated in all instances.

The investigation of the immune system has been discussed in detail in Chapter 7. Immunoglobulin levels may be assessed qualitatively by means of paper electrophoresis or immunoelectrophoresis, and can be measured quantitatively by a variety of techniques. Secretory immunoglobulins should also be examined. In addition, it is frequently useful to evaluate an individual's ability to produce antibodies towards specific bacterial and other antigens.

If reduced immunoglobulins are found, various primary and secondary immune deficiencies need to be considered. These are discussed in detail in Chapter 44. Causes of secondary hypogammaglobulinemia include chronic lymphocytic leukemia<sup>32</sup> (Chapter 49) which is easily diagnosed by its characteristic clinical and hematologic findings, and non-Hodgkin's lymphoma, which often requires a more diligent search (Chapter 51). The serum, urine, and bone marrow should be examined for evidence of one of the plasma cell dyscrasias (Chapters 52 and 53). Examination of the bone marrow is also useful in the investigation of primary defects in antibody production, since plasma cells are absent in many of them (Chapter 44).<sup>15</sup> When reduced immunoglobulins are due to renal protein loss, the nephrotic syndrome is usually present and is readily recognized. Protein-losing enteropathy also may lead to hypogammaglobulinemia, and sometimes gastrointestinal symptoms may be minimal.<sup>14</sup>

Defective cellular immunity may be accompanied by lymphopenia, as in severe

combined immune deficiency<sup>10,31</sup> or Hodgkin's disease<sup>1,30</sup> but the causes of lymphopenia are so varied (Chapter 41) that it is an unreliable index of defective cellular immunity. Reliable methods of assessing cellular immunity include: (1) the subcutaneous injection of antigen, which yields delayed hypersensitivity responses in the presence of immunity, (2) testing sensitization to contact allergens such as dinitrochlorobenzene (DNCB), and (3) a host of *in vitro* tests of lymphocyte function, including the response to phytohemagglutinin and specific antigens.<sup>19</sup> All of these tests and others have been discussed in detail in Chapter 7. The diseases causing secondary defects in cellular immunity are usually obvious; they include Hodgkin's disease, sarcoidosis, and certain advanced malignant lesions (Chapter 44).

If at this point in the patient's workup, no explanation for recurrent or persistent infections has been discovered, the possibility of a functional defect in the neutrophil system should be considered. Four classes of defects in neutrophil participation in inflammatory exudates have been described<sup>11</sup>: (1) intrinsic, but undefined, neutrophil defects resulting in markedly reduced ability of the cell to migrate from the blood to exudates; (2) normal neutrophils but defective chemotactic factors so that neutrophils are not attracted to exudates (Chapter 41); (3) defective killing of phagocytized organisms due to lysosomal enzyme deficiency; and (4) defective killing of phagocytized organisms due to as yet incompletely defined metabolic defects in the neutrophil. Neutrophil defects may be inherited or acquired, either as idiopathic syndromes or secondary to various diseases<sup>11</sup> (Chapter 42). Since all of these are newly defined classes of defects, their frequency is unknown. In order to test for their presence one must assess neutrophil migration into induced exudates<sup>7</sup> and test the ability of neutrophils to kill phagocytized organisms.<sup>11</sup> Since killing defects are sometimes limited to specific bacteria<sup>17</sup> the ability of the patient's neutrophils to kill his infecting organisms should be studied.

Finally, it is necessary to stop when a diag-

nosis has been made. For example, in a 60 year old man with symptoms limited to fatigue, physical examination which discloses generalized adenopathy and blood containing  $30.0 \times 10^9$  or more small lymphocytes/l, the diagnosis of chronic lymphocytic leukemia is established and bone marrow examination and lymph node biopsy provide no further diagnostic information. Too often, one sees patients in whom unnecessary biopsy procedures have been carried out.

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## *Variations of Leukocytes in Disease*

### Leukocytes in Inflammation

#### The Neutrophil Series

Causes of Neutrophilia

Causes of Neutropenia

Neutrophil Kinetics in Patients with Neutrophilia or Neutropenia

Estimation and Significance of Quantitative, Qualitative, and Morphologic Changes in Blood Neutrophils in Disease

Evaluation of the Functional Status of the Neutrophil System

#### The Eosinophil Series

Causes of Eosinophilia

#### The Basophil Series

#### The Monocyte Macrophage Series

Causes of Monocytosis

#### The Lymphocyte Series

Causes of Lymphocytosis

Lymphocytopenia

#### Plasma Cells

#### *Agranulocytosis and Drug Induced Neutropenia*

Agents Occasionally Associated with the Development of Leukopenia and Neutropenia

Symptomatology

Diagnosis

Prognosis and Course

Treatment

Pathology

#### Leukamoid Blood Pictures

Experimental Production of Leukemoid Reactions

ognized as measures of the reaction of the body to disease processes and noxious agents. In many instances these alterations give useful indications of the nature of the pathologic process, and they may be seen not only in acute infections but also in many chronic ailments as well.<sup>65 69,73</sup>

## **Leukocytes in Inflammation**

It was emphasized in Chapters 6 and 7 that the major leukocyte functions are accomplished in the tissues and that the leukocytes in the blood, even in normal persons, are in transit from sites of production or storage to the tissues. From this it is evident that variations in the blood concentration of each leukocyte type can result from changes in: (1) the flow of cells into the blood, (2) the egress of cells from the blood, (3) the distribution of cells within the vascular system, or (4) combinations thereof.<sup>30,10</sup> Furthermore, these changes may be of brief duration, and thus easily missed, or they may persist for days or weeks. Quantitative measurement of such changes in distribution and in inflow and egress rates has been possible only in recent years, and for only one or two of the leukocyte types (pages 249, 269). However, these studies have provided some insight concerning the pathophysiologic significance of the leukocyte variations seen in disease states.

Also helpful in gaining an understanding of the leukocyte responses noted in disease

Alterations in the blood leukocyte concentration and in the relative proportions of the several leukocyte types have long been rec-

have been a variety of *experimental models of inflammation*. These have included observations of cell migration into sites of implanted foreign bodies as carried out by Metchnikoff and of cell migration into rabbit ear chambers<sup>8</sup> and skin windows<sup>2,15,16,175</sup>; studies of peritoneal exudates,<sup>11</sup> hypersensitivity reactions,<sup>5,6</sup> and infections<sup>50</sup> and parasitic infestations<sup>252,253,261</sup> produced in animals; and investigation of the kinetics of labeled cells in human subjects given injections of endotoxin.<sup>33,55</sup> These observations and studies have been described in extensive reviews.<sup>5,6,7,10,18,20,21</sup> It has been shown that at sites of tissue damage, whether induced by a bacterial infection, the deposition of antigen antibody complexes, physical damage to cells (heat, freezing, irradiation, or chemicals), lysis of leukocytes as a result of their inability to digest urate crystals, or by some other means, a series of reactions referred to as the inflammatory process ensues. Although similar, these reactions are not identical in all instances and vary with the intensity, duration, extent, and type of injury.<sup>10,24</sup> In mild injuries there is an initial, transient vasodilatation of postcapillary venules accompanied by increased vascular permeability; this lasts less than 10 minutes and is thought to result from the local release of histamine.<sup>7,10</sup> There is increased blood flow to the area and a second wave of increased vascular permeability occurs during the 2- to 10-hour period following injury. This stage is accompanied by adherence of leukocytes to the vascular walls, most marked on the side nearest the injury,<sup>10</sup> and is mediated at least in part by kinins.<sup>7</sup> Diapedesis of white cells through the walls follows, neutrophils usually arriving first, with monocytes, eosinophils, basophils, and lymphocytes appearing later<sup>12</sup> in numbers that seem to be influenced by the nature of the injury.

In association with *small insults*, little change in the concentration of blood leukocytes occurs. However, if the lesion is extensive, large numbers of cells may be needed and are recruited from their sites of production or storage to enter the blood and thence to be transported to the tissues. In most bac-

terial infections, neutrophils ingest and usually kill the offending organisms, dying themselves soon thereafter. They usually are recruited in large numbers early in such infections. Macrophages ingest and remove damaged tissue cells, red cells, and neutrophils and, in general, appear to clean up the area of injury; they may even trigger the process of fibrosis and repair.<sup>1</sup> Macrophages do not usually die in performing these functions and, in fact, may be stimulated to divide at the site of inflammation, thus forming new cells.<sup>17,18</sup> Perhaps because of these properties, monocytes from which the macrophages arise appear to be recruited later than neutrophils and in smaller numbers. In some situations the neutrophils are unable to kill and digest certain foreign agents, eg, brucella, mycobacteria, toxoplasma, fungi. In these circumstances the macrophages become heavily involved in the phagocytosis and killing of the organisms and more than usual numbers are recruited for body defense. In most inflammatory lesions a few eosinophils and basophils are seen; in certain instances they are the prominent cell forms (see below). Lymphocytes appear late and in chronic lesions. In infections or trauma the inflammatory processes seem purposeful and result in protection of the organism. However, in other situations this is not always the case. For example, the ingestion of urate<sup>21</sup> or silica particles<sup>1</sup> by phagocytes results in phagosome lysis, death of the cell, and release of hydrolytic enzymes into the tissues. The release of enzymes also may occur in immune reactions of several types.<sup>5,6,21,22</sup> In these situations the activities of the leukocytes are harmful to the host and produce or exaggerate disease processes; in some of these, the suppression of leukocyte numbers and activities constitutes an effective form of therapy.<sup>5,13,21</sup> In any case the inflammatory reactions that take place in the tissues may produce changes in blood leukocyte content that are useful indicators of the nature of the disease and the host's response to it.

*Leukocytosis* refers to an increase above normal in the total number of leukocytes; that is, an increase above  $10.0 \times 10^9$  cells/l

(Table 6-4, page 242). In certain pathologic conditions, leukocyte counts of  $15.0$  to  $25.0 \times 10^9/l$  are common and values as high as  $40.0 \times 10^9$  cells/l are not unusual. Occasionally higher counts, even as high as  $100 \times 10^9$  cells/l may be found (see Leukemoid Blood Pictures, page 1301). Most commonly, leukocytosis is due to an increase in the number of neutrophils, and thus the term is often, but incorrectly, considered synonymous with *neutrophilia*. Less often leukocytosis may be due to an increase in lymphocytes (see page 1288). Leukocytosis due to eosinophilia, monocytosis, or basophilia is relatively rare.

*Leukopenia* refers to a decrease in the total leukocyte count below  $4.3 \times 10^9$  cells/l. Usually the reduction is due to a decrease in the number of neutrophils (*neutropenia*); however, when leukopenia is marked, lymphocytes and other cell types also are affected.

It must be appreciated that the concentration of several of the leukocyte types may change at the same time and in the same or in opposite directions. For example, in many acute infections the blood neutrophil concentration increases while there is a simultaneous decrease in lymphocytes and eosinophils. Significant changes in the less numerous cell forms, the eosinophils, basophils, and monocytes, may occur in the absence of changes in the total cell count. For these reasons and because it is now evident that each cell system has its own unique functions and control mechanisms it is best to determine the "absolute" blood concentration of each cell type (page 237). By this means, changes in the concentration of eosinophils, basophils, or monocytes will be detected as well as the more obvious changes in neutrophils and lymphocytes. Such information can provide significant clues to the alert physician.

## The Neutrophil Series

### Causes of Neutrophilia (Table 41-1)

ACUTE INFECTIONS. Neutrophilia may be due to acute infections, especially those caused by *cocci* (staphylococcus, strepto-

Table 41-1. Causes of Neutrophilia

- 1 *Acute infections*, local or generalized especially *coccal* but also those due to certain bacilli, fungi, spirochetes, parasites, and some viruses in diseases usually not associated with neutrophilia when complications develop
- 2 *Other inflammation* tissue damage resulting from burns or following operations, ischemic necrosis as in myocardial infarction, gout collagen vascular disease, hypersensitivity reactions, and other similar inflammatory processes
- 3 *Intoxication*
  - a Metabolic, including uremia diabetic acidosis, eclampsia
  - b Poisoning by chemicals and drugs lead, digitalis, insect venoms, foreign protein
- 4 *Acute hemorrhage*, internal, external
- 5 *Acute hemolysis*
- 6 *Malignant neoplasms*
- 7 *Physiologic neutrophilia* during strenuous exercise, after epinephrine injection, in association with convulsions or paroxysmal tachycardia and in the newborn
- 8 *Myelocytic leukemia*, polycythemia vera, myelofibrosis and myeloid metaplasia
- 9 *Other causes* chronic idiopathic neutrophilia, adrenocorticosteroids

coccus, pneumococcus, gonococcus, meningococcus) and by some bacilli (*E. coli*, *Pr. aeruginosa*, *C. diphtheriae*, *P. tularensis*), as well as certain fungi (*Actinomyces*), spirochetes (*L. icterohemorrhagica*), viruses (rabies, poliomyelitis, herpes zoster, smallpox, chickenpox), *rickettsia* (typhus) and *parasites* (liver fluke, coccidiosis immitis). It is found in association with *localized infections* such as furuncles, carbuncles, abscesses, tonsillitis, otitis media, and osteomyelitis, but often is most prominent in more *widespread infections* such as pneumonia, cholecystitis, salpingitis, meningitis, diphtheria, anthrax, plague, peritonitis, and appendicitis, to cite just a few.

In acute infections, leukocyte counts of  $15.0$  to  $25.0 \times 10^9$  cells/l are usually found. In pneumococcal pneumonia, higher counts ( $20.0$  to  $40.0 \times 10^9/l$ ) are characteristic and to some degree reflect the extent of the process; in bronchopneumonia a moderate leukocytosis is common.

The absence of leukocytosis is very helpful in differentiating typhoid fever, paratyphoid

fever and glands from pyogenic infections; leukocytosis also is usually absent in uncomplicated mumps, measles, and many viral infections. In these infections the presence of neutrophilia has been regarded as indicating the onset of complications, such as meningitis or orchitis in mumps, bowel perforation in typhoid fever, or bacterial infection in measles.<sup>65</sup> Nevertheless, leukocytosis has been observed in viral disease of the respiratory tract apparently in the absence of complicating infections<sup>68</sup> and is common in smallpox and chickenpox. In tuberculosis, neutrophilia is usually minimal or absent except in association with acute local spread, as in tuberculous meningitis or following rupture of caseous foci into the pleural space or into a bronchus.

**OTHER INFLAMMATION.** Leukocyte counts as high as  $73 \times 10^9$  cells/l have been reported in patients with severe burns; this is accompanied by a shift to the left and the presence of "degenerative" forms<sup>71</sup> including "toxic" granulation and Dohle bodies (page 1278). *Postoperatively*, neutrophilia occurs for 12 to 36 hours, perhaps as the result of the extensive tissue injury. In *coronary thrombosis*, neutrophilia regularly occurs and is valuable in distinguishing this condition from angina pectoris and more prolonged episodes of pain ("coronary insufficiency") that are not accompanied by tissue necrosis. Leukocytosis also occurs in intestinal obstruction and strangulated hernia, but many insist that it does not occur until peritonitis has set in. Neutrophilia commonly occurs during acute attacks of gout and may reach values of  $30.0 \times 10^9$ /l; this inflammatory reaction appears to result from the binding of uric acid crystals to phagosome walls, the rupture thereof, and finally the release of hydrolytic enzymes into the cell and then into the surrounding tissues.<sup>21</sup>

Neutrophilia occurs in acute glomerulonephritis, serum sickness, and rheumatic fever and in a number of the *collagen vascular diseases* that are thought to represent immune reactions. The appearance of neutrophils in certain types of hypersensitivity lesions (eg,

Schwartzman and Arthus reactions and experimental nephritis) suggests that a similar mechanism may be involved in these conditions.<sup>5,6</sup> This is yet to be proved. Of all the exanthems, leukocytosis is most marked in scarlet fever where counts as high as  $40.0 \times 10^9$ /l may be observed.

**INTOXICATIONS.** Intoxications include metabolic disorders and poisoning.

**METABOLIC DISORDERS.** In *ketoacidosis*, infections and other inflammatory lesions which may have precipitated the ketotic state should not be overlooked. Neutrophilia is especially prominent in the *uremic* patient with pericarditis or other inflammation related to the severe azotemia. The neutrophilic increase in *eclampsia* is poorly understood, but may in part reflect inflammation and in part may be caused by the convulsions.

**POISONING.** Poisoning by a variety of chemicals and drugs—lead, mercury, illuminating gas, potassium chlorate, digitalis, camphor, antipyrine, acetanilid, phenacetin, pyridine, pyrogallol, turpentine, benzene derivatives, arsphenamine—as well as by insect venoms, such as the venom of the black widow spider, may result in leukocytosis and neutrophilia. The response is almost certainly related to the degree of tissue necrosis produced and whether or not vomiting, convulsions, or a hypersensitivity reaction develops. A number of the above-mentioned drugs have also been found to cause a reduction in the leukocyte count, especially in the number of neutrophils. This effect may depend on the degree and nature of the poisoning, leukocytosis occurring when the effect is temporary or less serious. In *lead colic*, counts as high as  $20.0 \times 10^9$  leukocytes/l may be found; muscular activity may account for the leukocytosis in this situation and in delirium tremens. The injection of *foreign protein*, such as typhoid vaccine or endotoxin, produces a transient decrease in leukocyte count which occurs one and two hours after injection followed by an increase in leukocyte count (Fig. 41-5). Similar reactions are noted in serum disease and during the Jarisch-Herxheimer reaction.<sup>66,70</sup>



**ACUTE HEMORRHAGE.** Leukocytosis develops within an hour or two following the onset of acute hemorrhage.<sup>67</sup> It is greater when the bleeding occurs into the peritoneal cavity, the pleural space, a joint cavity, or adjacent to the dura than when the bleeding is external. In ruptured tubal pregnancy the count may be as high as  $22.0 \times 10^9$  cells/l. When the skull is fractured and there is associated intracranial bleeding or in subarachnoid hemorrhage, even higher counts are sometimes found. A value as high as  $31.0 \times 10^9/l$  has been observed following rupture of the spleen. The mechanism by which leukocytosis and neutrophilia develop in such cases is poorly understood. Since small volumes of blood may cause marked neutrophilia in some instances, pain with release of adrenal corticosteroids and/or epinephrine may be involved. In addition, local inflammation, perhaps due to pressure necrosis and/or the lysis of leukocytes with release of hydrolytic enzymes, may also contribute, possibly by generating chemotactic factors (page 257). Large external hemorrhages also cause neutrophilia, but in these the rate and amount of blood lost seem to be important factors<sup>67</sup>; the lack of eosinopenia in phlebotomized dogs militates against an adrenocorticoid mediated mechanism.<sup>67</sup>

**ACUTE HEMOLYSIS.** In mismatched transfusions, inadvertent infusion of hypotonic solutions, or acute hemolytic diseases, marked leukocytosis may occur.

**MALIGNANT NEOPLASMS.** Rapidly growing neoplasms may cause neutrophilia, presumably as a result of necrosis within areas that have outgrown their blood supply. When the liver, gastrointestinal tract, or bone marrow are involved, the counts may be especially high (see Leukemoid Blood Pictures). There is also evidence in animals that some tumors may contain hormone-like substances that cause leukocytosis (page 1303).

**PHYSIOLOGIC NEUTROPHILIA.** Transient neutrophilia occurs in association with vigorous exercise or after the injection of epinephrine<sup>33</sup> (page 249). Similarly, neutrophilia

occurs in association with convulsions and in paroxysmal tachycardia and during labor. It is also seen in the newborn.

**CHRONIC MYELOCYTIC LEUKEMIA AND POLYCYTHEMIA VERA.** In chronic myelocytic leukemia neutrophilia is associated with a marked "shift to the left" with metamyelocytes, myelocytes, and even younger forms in the blood, while in polycythemia vera there is usually little or no shift to the left. A few leukemia patients have been observed in whom the predominating cells in the blood were polymorphonuclear neutrophils (see Chapter 48).

**OTHER CAUSES.** Rarely, neutrophilia, with total leukocyte counts as high as  $43.0 \times 10^9$  cells/l, has been observed in the absence of any of the above disorders, and has persisted for a long time without a cause being discovered.<sup>72</sup>

The production of neutrophilia by the acute or chronic administration of adrenocorticosteroids is well recognized.<sup>33,37</sup> Neutrophilia is commonly seen in Cushing's disease.

### Causes of Neutropenia (Table 41-2)

**ACUTE INFECTIONS. BACTERIAL INFECTIONS.** Typhoid and paratyphoid fever and sometimes tularemia are commonly mentioned as acute infections accompanied by leukopenia. In typhoid fever, considerable variation in total leukocyte count occurs, with values ranging from  $1.9$  to  $25.0 \times 10^9$  cells/l.<sup>102</sup> A total leukocyte count greater than  $10.0 \times 10^9$  cells/l was reported in only 9% of 189 typhoid fever patients, while neutropenia was present in 47%.<sup>115</sup> Early in the disease (first week), mild leukocytosis and neutrophilia may be present,<sup>102</sup> but polymorphonuclear numbers then decrease to a minimum when bacteremia appears.<sup>81,102</sup> Minimum neutrophil concentrations seldom fall below  $0.6 \times 10^9$  cells/l,<sup>106</sup> but agranulocytosis has been reported.<sup>81</sup> The paratyphoid fevers (salmonellosis) produce a similar clinical picture.<sup>115,119</sup> In tularemia of the typhoidal or pulmonary type, absence of leukocytosis also

is the rule.<sup>80,82,138</sup> In a series of 225 patients with varying types of tularemia the average leukocyte concentration was 11.2 (range 2.0 to  $90.0 \times 10^9$  cells/l), but in only five patients was the leukocyte count less than  $5.0 \times 10^9$  cells/l.<sup>132</sup> In a series of 888 patients with *brucellosis*, most of whom had the severe and acute form of the disease, leukocyte counts less than  $4.5 \times 10^9$  cells/l were noted in 144<sup>93</sup>; even in those patients with leukopenia, neutropenia was minimal.

**VIRAL INFECTIONS.** A wide variety of viral diseases produce leukopenia and neutropenia. The best documented are yellow fever (acquired accidentally in the laboratory<sup>86</sup>), experimentally induced sandfly fever,<sup>137</sup> and infectious hepatitis.<sup>111</sup> In these diseases a gradual fall in leukocyte count begins usually during the second day of fever and reaches a nadir between the fourth and sixth days (Figs. 41-1 and 41-2); this fall reflects a decrease in both neutrophils and lymphocytes, neutropenia being more prominent in some viral infections and lymphopenia in others; counts may or may not reach levels of absolute neutropenia or absolute lymphopenia. In sandfly fever (Fig. 41-1), over 90% of patients develop leukopenia, and an absolute neutropenia with an increase in immature neutrophils is said to be a constant finding.<sup>137</sup> In infectious hepatitis, both neutropenia and lymphopenia are common and precede the onset of icterus (Fig. 41-2); with the rise of leukocyte concentration toward normal, atypical lymphocytes appear in the blood.<sup>111,123a</sup> In yellow fever, leukopenia of 1.5 to  $2.5 \times 10^9$  cells/l with neutropenia of 0.5 to 1.0 and also lymphopenia are common, usually occurring on about the fifth day.<sup>86</sup> Many other acute infections caused by viruses are characterized by such changes<sup>101</sup>; these include measles either naturally acquired<sup>85</sup> or occurring after inoculation with live vaccine,<sup>91</sup> chickenpox,<sup>114</sup> rubella,<sup>112,125</sup> Colorado tick fever,<sup>116</sup> dengue, and hemorrhagic fever.<sup>90</sup> Leukopenia sometimes occurs in *infectious mononucleosis*<sup>128</sup> (Chapter 43). Depletion of mature neutrophils in the marrow has been reported in *Colorado tick fever*<sup>116</sup> and

**Table 41-2. Causes of Leukopenia and/or Neutropenia**

- 1 *Certain infections*
  - a *Bacterial* typhoid, paratyphoid, less often brucellosis, rarely tularemia
  - b *Viral* eg, influenza, measles, infectious hepatitis, chickenpox, rubella, Colorado tick fever, dengue, yellow fever, sandfly fever
  - c *Rickettsia* rickettsial pox, typhus, Rocky Mountain spotted fever
  - d *Protozoal* malaria, kala azar, relapsing fever
- 2 All types of overwhelming infections eg miliary tuberculosis, septicemia, especially in debilitated patients with poor resistance
- 3 *Effect of physical agents, chemicals, and drugs*
  - a Chemical and physical agents that produce marrow hypoplasia and aplasia in all subjects if given in sufficient dose, eg, ionizing radiation, benzene, nitrogen mustards, urethane, antimetabolites (eg, folic acid antagonists, purine and pyrimidine analogues), periwinkle alkaloids (eg vincblastine) colchicine
  - b Chemicals and drugs that occasionally produce leukopenia apparently as a result of individual sensitivity eg aminopyrine, phenothiazines, sulfonamides, antithyroid drugs, anticonvulsants, antihistamines, tranquilizers, antimicrobial agents
- 4 *Certain hematologic and other conditions of unknown or poorly defined etiologic basis*
  - a Those that may be due to decreased or ineffective production, eg pernicious anemia, aplastic anemia, chronic hypochromic anemia
  - b Those that may be due to increased utilization or destruction eg cirrhosis of the liver with splenomegaly, lupus erythematosus, Felty's syndrome, Banti's syndrome, Gaucher's disease
- 5 Cachexia and debilitated states (alcoholism, etc)
- 6 In anaphylactoid shock and in early stages of reaction to foreign protein
- 7 Certain rare hereditary, congenital or familial and miscellaneous disorders (cyclic neutropenia, chronic hypoplastic neutropenia, infantile genetic agranulocytosis, primary splenic neutropenia) (see Chapter 42)

in *hemorrhagic fever*<sup>90</sup>; this suggests increased utilization or direct damage to polymorphonuclear cells as possible mechanisms of the blood neutropenia. The shift to the left often noted in the blood differential count and the presence of toxic granulation provide further support for this thesis (also see section on

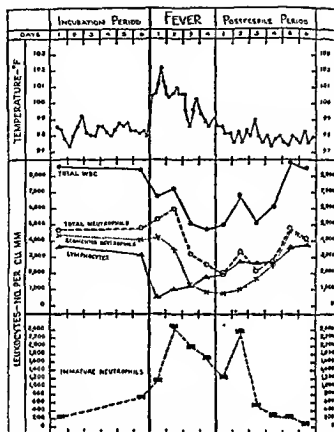


Fig 41-1. The changes in the total leukocyte neutrophil, and lymphocyte concentration during experimentally induced sandfly (Phlebotomus) fever (From Sabin et al<sup>117</sup> courtesy of the authors and JAMA)

kinetics of the inflammatory reaction, page 1275)

**RICKETTSIA** Leukopenia and neutropenia may occur with variable frequency in most rickettsial infections, usually during the first week of the disease. Leukopenia is very common in *rickettsialpox*,<sup>134</sup> less so in *epidemic typhus*,<sup>144</sup> scrub typhus,<sup>103</sup> Rocky Mountain spotted fever,<sup>110</sup> and recrudescent typhus.<sup>94</sup>

**PROTOZOAL INFECTIONS** In *malaria*, slight leukocytosis ( $11.0 \times 10^9/l$ ) may be present for a short time during paroxysms, but, as parasitemia progresses, leukopenia<sup>130</sup> and shift neutropenia<sup>100</sup> develop. In relapsing fever the leukopenia is found between periods of fever; during febrile periods, the leukocyte count may be as high as  $15.0 \times 10^9$  cells/l. Leukopenia also characterizes kala-azar<sup>96</sup> and oriental sore.

**OVERWHELMING INFECTION.** Neutropenia may result from overwhelming infection of all types, eg, miliary tuberculosis<sup>84</sup> and septicemia<sup>88</sup> (Fig. 41-3). Patients with depressed marrow neutrophil reserves are particularly prone to develop neutropenia when severe infection ensues. Such patients include alcoholics,<sup>127</sup> the undernourished, and patients with primary hematologic disease or those previously treated with marrow-depressing drugs or irradiation. However, even the previously healthy may develop severe neutropenia when overwhelming infections develop<sup>88</sup> (Fig. 41-3).

**CHEMICAL AND PHYSICAL AGENTS.** Certain chemical and physical agents may produce leukopenia and granulocytopenia. These may be divided into two general categories: those causing leukopenia and neutropenia consist-

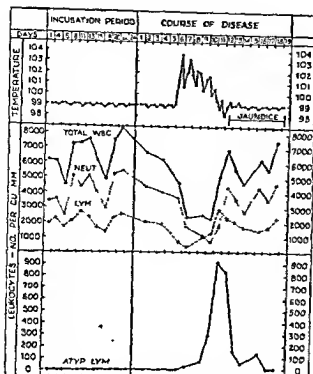


Fig 41-2 The changes in total leukocyte neutrophil and lymphocyte concentration during experimentally induced infectious hepatitis (From Havens and Marck<sup>111</sup> courtesy of the authors and the American Journal of Medical Sciences)

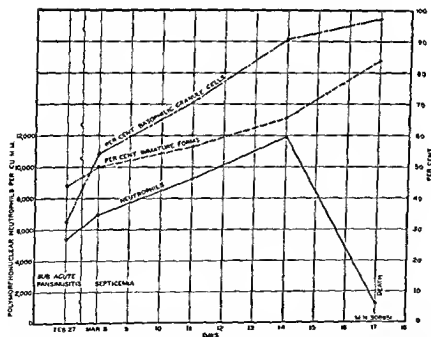


Fig 41-3 The blood neutrophil pattern in a previously healthy woman without other disease who developed acute pansinusitis which progressed to cavernous sinus thrombosis and terminated in streptococcal septicemia. The total neutrophil response was inadequate and the large proportion of immature forms and cells containing toxic granulations indicate an inadequate response (From Bethell,<sup>88</sup> courtesy of the author and Journal of Laboratory and Clinical Medicine)

ently and those occasionally associated with neutropenia.

**AGENTS THAT CAUSE LEUKOPENIA AND NEUTROPENIA CONSISTENTLY** If given in sufficient dose certain agents will consistently cause leukopenia and neutropenia (Table 41-2). For the most part these are agents used in the chemotherapy of malignant disease. They act mainly, if not entirely, by interfering with cell production, either by directly damaging the mitotic or stem cell compartments of the marrow (ionizing radiation, benzene) or by slowing cell division in various ways such as: (1) interfering with the purine or pyrimidine biosynthetic steps necessary for DNA synthesis (antimetabolites); (2) blocking DNA strand duplication as a result of drug binding to certain base groups in the DNA molecule (mustard analogues); (3) disrupting the microtubules of the mitotic spindle (colchicine, vinblastine, vincristine); or (4) interfering with RNA formation and the translation and transcription processes that are central to protein synthesis in all cells (certain antibiotics). Since most of these agents also suppress erythrocyte and platelet production they are discussed further in Chapters 34 and 56.

Generally, after exposure to agents of this type, the degree of neutropenia is related to dose. Also there is a variable delay before the deleterious effects on cell production are reflected in the appearance of neutropenia.<sup>93</sup> The length of the delay is related to the size of the marrow neutrophil reserve before the agent is given and the rate of consumption of cells thereafter. Thus, after complete cessation of cell production in a previously healthy person and in the absence of an increased need for neutrophils, a delay of 8 to 14 days can be expected before neutropenia will develop (page 249). On the other hand, in a chronically ill patient who may have been exposed to these agents previously, whose marrow may contain tumor, or whose reserves have been depleted for nutritional or other reasons, the interval between exposure and neutropenia may be shorter. The presence of increased neutrophil demand (infection or other inflammation) also will shorten the interval.

Unless damage to the mitotable precursors or the stem cells has been excessive, evidence of regeneration in the marrow often is apparent by the time neutropenia develops.<sup>93</sup>

**AGENTS OCCASIONALLY ASSOCIATED WITH NEUTROPENIA** (Table 41-2). These agents cause neutropenia that may be of acute onset and apparently the result of hypersensitivity (page 1292), or it may consist of a gradual reduction in cell concentration over an extended time; the mechanism of the latter process is not well understood. These neutropenias will be discussed later in this chapter (page 1292).

**HEMATOPOIETIC DISORDERS AND CERTAIN CONDITIONS OF UNKNOWN CAUSE.** Patients with chronic anemias, such as pernicious anemia, may be leukopenic and/or neutropenic, presumably because cell production is markedly impaired or "ineffective." Rarely, leukopenia or neutropenia is seen in chronic hypochromic anemia.<sup>118</sup>

Leukopenia and neutropenia also may occur in certain other conditions of poorly understood pathogenesis (Table 41-2). In patients with *cirrhosis of the liver*, especially in those with splenomegaly (page 1412),<sup>39,48,126</sup> there is a tendency to leukopenia, granulocytopenia, and monocytosis.<sup>126</sup> Leukopenia occurred in 66% of 258 patients with *lupus erythematosus*.<sup>99</sup> In *Felty's syndrome*, a condition characterized by arthritis, splenomegaly, and leukopenia, not only is there a variable reduction in concentration of neutrophils and total cell count,<sup>122,135</sup> but also increased susceptibility to infection is not uncommon (page 1408).

**CACHEXIA AND DEBILITATED STATES.** Cachexia and debilitated states associated with protein undernutrition and vitamin deficiency are accompanied by leukopenia.<sup>105</sup> Leukopenia and neutropenia are not seen in alcoholics in the absence of nutritional deficiency.<sup>120</sup>

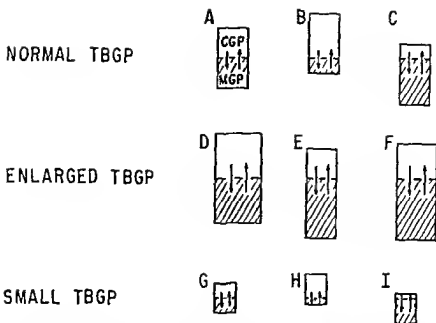


Fig 41-4 Variations in blood neutrophil pool size and the distribution of cells in marginal and circulating sites that occur in patients with a variety of diseases. TBGP is the total granulocyte pool, CGP, circulating granulocyte pool, MGP, marginal granulocyte pool. The normal TBGP size and equal distribution of cells between MGP and CGP are illustrated in A, the continuous exchange of cells between CGP and MGP being depicted by the arrows. Alterations from normal are shown in B through I. B depicts "shift neutrophilia." C shift or "pseudo" neutropenia. D illustrates an absolute neutrophilia with increased TBGP size and equal distribution of cells in CGP and MGP. F is similar but with a greater proportional increase in MGP size. E illustrates masked granulocytosis: an increase in TBGP size without neutrophilia.

C, G, H, and I illustrate neutropenic states. C depicts shift or pseudoneutropenia, the TBGP being normal. CGP decreased. G, H, and I illustrate absolute decreases in TBGP size with different distributions of cells between marginal and circulating sites, in G the neutrophil concentration reflects the reduction in TBGP size because cells are evenly distributed in CGP and MGP. However, in H the neutropenia is more severe than the blood count indicates. In I the reverse is the case. (From Athens,<sup>31</sup> courtesy of the author and Appleton-Century Crofts.)

**ANAPHYLACTOID SHOCK.** Neutropenia is associated with anaphylactoid shock and the early stage of the reaction to parenterally introduced foreign protein or endotoxin. In sensitized animals the reinjection of the sensitizing protein results in anaphylaxis and massive accumulation of neutrophils in the lungs. During the second hour after the injection of typhoid vaccine or purified endotoxin a similar transient neutropenia develops (Fig. 41-5). The latter event apparently is due to margination of neutrophils and is soon followed by neutrophilia.

**RARE DISORDERS.** Certain additional rare disorders of which neutropenia is an important feature will be discussed in Chapter 42.

#### Neutrophil Kinetics in Patients with Neutrophilia or Neutropenia<sup>30,31,40</sup>

It has been demonstrated by means of infusions of autologous,  $DF^{32}P$ -labeled neutrophils (page 249) that an increase in blood neutrophil concentration may reflect either a shift of cells from marginal sites into the circulating granulocyte pool (CGP) without a change in total blood granulocyte pool size (TBGP) ("shift neutrophilia" [Fig. 41-4B]) or a true increase in TBGP size ("true neutrophilia" [Fig. 41-4D and F]). "Shift neutrophilia" occurs after active exercise or epinephrine injection<sup>32,33</sup>; it is of brief duration, usually lasting less than 20 to 30 minutes. It has been proposed that a similar mechanism

may explain the neutrophilia occurring after seizures or in association with paroxysmal tachycardia,<sup>32</sup> but no experimental documentation of this exists. Shift neutrophilia is usually moderate in degree ( $<15.0\text{--}20.0 \times 10^9$  neutrophils/l) and characteristically there is no increase in nonsegmented forms; that is, there is no change in inflow of neutrophils into the blood from the marrow or in egress of neutrophils from the blood.<sup>33</sup>

*True neutrophilia* is characterized by an increase in blood neutrophil concentration above  $7.25 \times 10^9$  cells/l (Table 6-4, page 242) and an increase in TBGP size above  $160 \times 10^7$  granulocytes/kg (Table 6-5, page 250). Most cases of neutrophilia due to *chronic infections* or a variety of miscellaneous diseases fall into this category (Fig. 41-4D and F). The neutrophils are predominantly mature and the TBGP size ranges from normal to five or six times normal. The distribution of cells between CGP and MGP varies considerably.<sup>35</sup>  $T_{1/2}$  values in such patients are normal or slightly prolonged and the blood granulocyte turnover rate (GTR), a measure of effective cell production, ranges from high normal values to 12 times normal.<sup>33</sup> In patients with *acute infections* the findings are similar, but it has been impossible to study patients early in infection.<sup>30</sup> From studies of two models of acute infection, namely, endotoxin injection in man<sup>31,35</sup> and bronchopneumonia produced in dogs,<sup>30</sup> it has been inferred that the changes in neutrophil kinetics can be divided into early, intermediate, and late phases. During early infection there may be a brief period during which the neutrophil concentration decreases, perhaps even to neutropenic levels, owing to margination of cells.<sup>33</sup> During this phase there is no decrease in TBGP size and, in fact, it may be somewhat increased.<sup>30,33</sup> The period of margination is rapidly followed by an influx of cells from the bone marrow into the blood<sup>30,35</sup> and an increase in TBGP size and blood neutrophilia<sup>33,30</sup>; during this early phase the  $t_{1/2}$  of labeled neutrophils is shorter than normal.<sup>50</sup> In contrast to the picture most common in chronic infections, if the demand for cells is great enough a shift to the left in the differential count occurs.<sup>50</sup> The

intermediate phase (established inflammation) is characterized by elevated neutrophil concentration in the blood and enlarged TBGP, usually with approximately equal numbers of cells in the MGP and CGP. If the infection is controlled, normal or somewhat long  $t_{1/2}$  values<sup>30,50</sup> and increased GTR<sup>35</sup> are found, but if the demand for cells is very great the marrow stores may become exhausted and neutropenia ensues.<sup>50</sup> During the late phase (recovery) there is a decreased flow of marrow neutrophils into the blood and a prolonged  $t_{1/2}$  as the neutrophilia subsides.<sup>50,55</sup>

Studies in which neutrophil precursors in the marrow were labeled with tritiated thymidine<sup>44</sup> or radiophosphate<sup>37</sup> also demonstrated more rapid release of cells to the blood in these conditions.

During the steady-state neutrophilia seen in patients with *polycythemia vera* or *myelofibrosis*, the TBGP has been found to be enlarged but an increased proportion of cells is located in the MGP (Fig. 41-4E and F) so that the neutrophil concentration does not truly reflect the increase in TBGP size.<sup>35</sup> In some patients the neutrophil concentration actually was normal although the TBGP was enlarged due to the presence of a large MGP (Fig. 41-4E). In patients with predominantly mature neutrophils in the blood the  $t_{1/2}$  values ranged from normal to 18 hours and the GTR ranged from normal to 12 times normal.<sup>35</sup> In patients with *chronic myelocytic leukemia* and in patients with myelofibrosis or myeloid metaplasia in whom increased numbers of immature neutrophils (metamyelocytes and younger forms) were present in the blood, the TBGP also was increased in size, but the  $t_{1/2}$  values were markedly prolonged (25 to 90 hours),<sup>34,46,51</sup> presumably due to the inability of immature cells to leave the blood readily<sup>39</sup> and/or because of the recirculation of immature cells to the bone marrow and spleen.<sup>54,58</sup>

An increase in TBGP size due to selective enlargement of the MGP may occur in the absence of neutrophilia and is illustrated in Figure 41-4E. This situation has been referred to as "*masked*" *granulocytosis*<sup>41</sup> and has been encountered in occasional patients with

chronic infection, Hodgkin's disease, or polycythemia vera,<sup>35</sup> in the early phase of post-endotoxin neutrophilia<sup>33</sup> and in patients with induced skin inflammations.<sup>41</sup>

A single injection of hydrocortisol produces a transient neutrophilia of a few hours' duration (Fig. 41-5) that is due partly to an increased rate of neutrophil release from the bone marrow and partly to a decrease in egress of cells from the blood.<sup>37</sup> Continued oral administration of a glucocorticoid such as prednisone results in a stable neutrophilia with an enlarged TBGP. The  $t_{1/2}$  values are normal or slightly prolonged (9 to 13 hours) and neutrophil production and destruction (GTR) are maintained at normal rates.<sup>33</sup> The movement of neutrophils into sites of inflammation nevertheless remains decreased.<sup>40,39</sup> It is postulated that this may in part explain the apparent increased susceptibility to infection of patients receiving steroids and perhaps contributes to the beneficial effects of steroids in gout, collagen vascular disease, and hypersensitivity reactions.

Fewer studies have been carried out in neutropenic states because of technical difficulties. Nevertheless, it is evident that patients may have a shift neutropenia or a true neutropenia. In "shift" or "pseudoneutropenia" the blood neutrophil concentration is decreased, but the TBGP remains normal (Fig. 41-4C).<sup>35,40</sup> Shift neutropenia may be transient in character, as after endotoxin<sup>33</sup> or nicotinic acid<sup>36</sup> injection, or constant, as in chronic neutropenic states.<sup>38</sup> However, in most patients with neutropenia there is a reduction in TBGP size; this is *true neutropenia*, the size of both the CGP and MGP being decreased (Fig. 41-4G, H, and I) although not always proportionately so.<sup>38,49</sup> On the basis of kinetic studies, true neutropenia may result from decreased cell production with normal cell survival in the blood or from decreased cell survival (short  $t_{1/2}$  values).<sup>38,42,48,52</sup> Neutropenia due to decreased cell production is associated with marrow suppression by drugs or x-ray therapy,<sup>38,52,60</sup> malignant disease involving the bone marrow such as leukemia and multiple myeloma,<sup>38,43</sup> and occasional cases of lupus erythematosus or cirrhosis.<sup>38</sup> Neutropenia

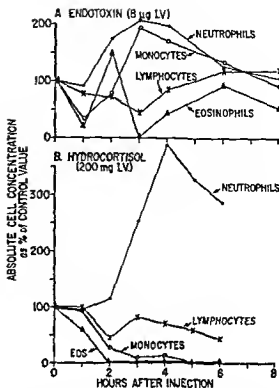


Fig 41-5 The effects on the concentration of blood leukocytes of intravenous endotoxin compared with those of intravenous hydrocortisol. At time zero a normal subject was given (A) 8 µg of endotoxin intravenously, one week later the same subject was given (B) 200 mg of hydrocortisol phosphate intravenously.

due to increased cell destruction (short  $t_{1/2}$ ) may be associated with subnormal, normal, or increased neutrophil production in the marrow<sup>38,52</sup> and occurs in a variety of diseases, including cirrhosis with splenomegaly, lupus erythematosus, megaloblastic anemia, viral infection, Felty's syndrome, and other conditions.<sup>38,52</sup> The mechanisms by which cell survival is shortened and marrow production is altered in these states are not yet clear. In some patients, splenectomy has alleviated both the neutropenia and the frequent infections,<sup>107</sup> and the kinetic picture has returned toward normal.<sup>33,52</sup>

#### Estimation and Significance of Quantitative, Qualitative, and Morphologic Changes in Blood—Neutrophils in Disease

Long before kinetic and other studies provided greater insight regarding the pathologic



physiology of alterations of leukocytes in disease, experienced clinicians, by paying careful attention to quantitative and qualitative changes in leukocytes, were able to draw inferences of value in judging the nature of disease processes and their probable outcome. It was recognized, for example, that pyogenic bacteria usually call forth a neutrophilic response the magnitude of which depends on: (1) the virulence of the organism, (2) whether the infection is localized or widespread, and (3) the patient's ability to respond to the process. Thus neutrophilia was noted to be minimal or absent when the pyogenic infection was mild, while a brisk neutrophilia in a patient with serious infection indicated good resistance. In serious infection, successive detailed blood examinations were found to be of considerable value prognostically; eg, a low or falling count and a shift to the left with metamyelocytes and myelocytes appearing in the blood suggested exhaustion of marrow neutrophil reserves and a poor outcome.<sup>89,127</sup>

It was also recognized that toxic granulation (see below), cytoplasmic vacuoles,<sup>223</sup> diffuse cytoplasmic basophilia,<sup>224</sup> pyknotic areas in the nucleus,<sup>214</sup> and Dohle bodies<sup>229</sup> (see below) appear in severe infections and have grave prognostic significance. The presence of one or more of these changes<sup>199,214,221,231</sup> and especially of toxic granulation suggests the development of bacteremia and generalized infection as opposed to a localized one.<sup>199,224</sup> The *degenerative index* was introduced as a means of quantitating these changes.<sup>199,214,224</sup> An index greater than 50% (more than 50% neutrophils exhibiting toxic granulation) suggested severe infection and a grave prognosis; serial indices were found to be more informative than changes in leukocyte concentration or a left shift in the differential count.<sup>199</sup> More recently the presence of *cytoplasmic vacuoles* in blood neutrophils has been found to correlate well with the presence of bacteremia<sup>231</sup> and impaired granulocyte function.<sup>223</sup>

Kinetic studies have confirmed many of these clinical impressions,<sup>50</sup> and have demonstrated that the magnitude of the blood

neutrophil concentration is affected both by the rate of cell inflow from the marrow and the rate of cell egress into the tissues. They indicate, furthermore, why the magnitude of the neutrophil count is not by itself a wholly reliable index of prognosis. For example, in a patient with extensive infection who is responding well, a high blood neutrophil count results because cell inflow from a well-stocked marrow is exceeding cell outflow into the infected tissues. On the other hand, very high neutrophil counts ( $40.0 \times 10^3$  cells/l) occur in the agonal state and appear to be related to terminal rises in plasma steroid level<sup>229</sup>; here the high steroid level enhances mobilization of marrow neutrophils, but blocks their egress from the blood into the tissues.<sup>37</sup> In the neutropenia seen in overwhelming infection there is a continued need for and egress of cells into the tissues, but the exhausted marrow is unable to supply enough cells to keep up with the demand.<sup>50</sup>

In contrast to the pyogenic infections, leukopenia and neutropenia are commonly associated with some bacterial (page 1270) and viral infections (page 1271) and are not necessarily attended with a poor outcome. The pathophysiology in these conditions is yet to be studied, however. Since, in typhoid fever, chills produced by cold baths resulted in neutrophilia,<sup>141</sup> it seems possible that the neutropenia characteristic of typhoid fever reflects a shift of neutrophils into a large marginal neutrophil pool; however, this remains to be proved.

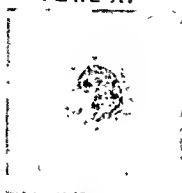
### Toxic Granules (Fig. 41-6) (Plate XVI)

Toxic granules were thought either to reflect a disturbance in neutrophil maturation with persistence of azurophilic granules even into mature cell stages<sup>199</sup> or to be the result of absorption of toxic agents that resulted in new abnormal granule formation.<sup>187</sup> Since toxic granulation, vacuole formation, increased cytoplasmic basophilia, and other changes can be produced by incubating neutrophils in vitro,<sup>187</sup> it seemed highly probable that these changes are induced in vivo in already formed neutrophils, and perhaps rep-

# PLATE XV



A



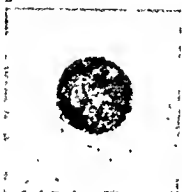
B



C



D



E



F



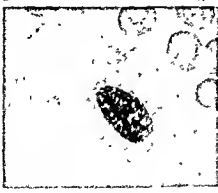
G



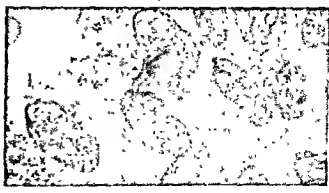
H



I



J



K

Miscellaneous cells and stains (photomicrographs,  $\times 1000$ )

A-E, Leukocyte alkaline phosphatase, stain A = 0, B = 1+, C = 2+, D = 3+, E = 4+.

F-I, LE cells (F, G) and "tart" cells (H, I).

J and K, Extraneous cells found in blood films J, epithelial cell, K, endothelial cells, probably from a vein

resent phagosomes or autophagic vacuoles. However, electron microscopy and histochemical studies now have shown that toxic granules are azurophilic (primary) granules that are peroxidase positive and are more readily stained than are the granules in normal cells.<sup>198</sup> An increase in non-granule-associated acid phosphatase in cells containing toxic granules suggests that there has been some release of azurophilic granule enzymes.<sup>198</sup> This finding and the increase in alkaline phosphatase activity that may also be seen in infection (see below) imply that toxic neutrophils are "stimulated" perhaps in a manner similar to that in which cells involved in phagocytosis or reacting to treatment with endotoxin are stimulated (page 259).

### ***Döhle Bodies (Fig. 41-6)***

Döhle bodies are discrete, round or oval areas seen in the peripheral portions of the cytoplasm of neutrophils; they stain sky-blue with Romanowsky dyes and have been identified by electron microscopy to be lamellar aggregates of rough endoplasmic reticulum (Fig. 41-6E).<sup>198</sup> They were first described as present in patients with scarlet fever but are also found to be associated with many infections, severe burns,<sup>229</sup> exposure to cytotoxic agents,<sup>198</sup> and uncomplicated pregnancy.<sup>150</sup> They are similar to and must be differentiated from May-Heggelin bodies (page 1322 and Fig. 42-4).

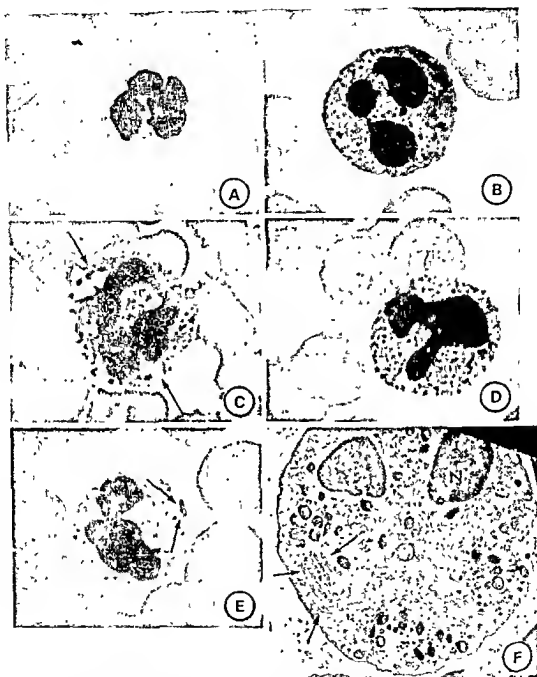
### ***Tests for Enzyme Activity***

Since there is increased intracellular enzyme activity in association with phagocytosis (page 259), measurements of such activities may prove clinically useful. Thus, in the *NBT dye test* the spontaneous reduction of colorless, nitro blue tetrazolium dye to blue-black formazan deposits within neutrophils demonstrates the presence of NADH-oxidase activity (page 1328 and Fig. 42-6). In normal persons, less than 10% of blood neutrophils are NBT positive.<sup>196,211</sup> In most patients with bacterial infections and bacteremia, more than 10% of neutrophils are NBT posi-

tive, while in those with a wide variety of other febrile illnesses, including viral infections, tuberculosis, and post-perfusion syndrome, normal values have been found.<sup>181,182,196,210</sup> Thus, the NBT test may be useful in differentiating certain types of bacterial infection from other illnesses. It is also helpful in the diagnosis of chronic granulomatous disease (page 1328).

*Leukocyte alkaline phosphatase (LAP)*<sup>169</sup> is capable of hydrolyzing phosphorus from a wide variety of phosphomonoesters. Activity of this enzyme has been shown by both histochemical (Plate XV) and biochemical methods to be moderately increased in neutrophil leukocytes in physiologic leukocytosis, pyogenic infection, and leukemoid reactions, but it also is increased in Hodgkin's disease, myocardial infarction, pregnancy, undelivered hydatidiform mole (in contrast to choriocarcinoma),<sup>186</sup> and in "stressful" conditions, and can be increased by the administration of ACTH and adrenocorticosteroids to normal individuals. As a rule, in polycythemia vera (in contrast to secondary forms of polycythemia),<sup>208</sup> some cases of myelofibrosis, and idiopathic thrombocytopenia greatly increased activity is found,<sup>193</sup> whereas low levels of activity usually characterize chronic myelocytic leukemia, but this is not always the case.<sup>191,225</sup> Remission may be associated with a return to normal values. Higher than normal values were reported in acute lymphoblastic leukemia in contrast to zero values in acute myeloblastic leukemia. In chronic lymphocytic leukemia, lymphosarcoma, and reticulum cell sarcoma, values within or just above or below the normal range were found.<sup>189</sup>

Low levels of LAP also have been reported in individuals with paroxysmal nocturnal hemoglobinuria and in a few with myelofibrosis. They have been found associated with infectious mononucleosis, pernicious anemia, aplastic anemia, thrombocytopenic purpura, and sarcoidosis,<sup>169</sup> as well as with the metabolic disorder, hypophosphatasia. Androgenic hormones may inhibit this enzyme.<sup>219</sup> LAP is increased in mongolism, a condition associated with trisomy of chromosome 21.<sup>154</sup>



**Fig. 41-6** A Normal blood neutrophil stained with Wright's stain (X2000) B, Normal blood neutrophil stained for 60 minutes with Wright's stain (X2120) Note that, compared with A, the granules are deeply stained and appear similar to those seen in toxic cells C, Cytoplasmic vacuolization (arrows) in toxic neutrophil (X2120) D Toxic granulation in a band neutrophil from a blood smear of a bacteremic patient, stained with Wright's stain for four minutes Note the similarity between the cytoplasmic granulation in this cell and that in B E Dohle body (arrows) in blood neutrophil stained for four minutes with Wright's stain (X2120) F, Electron micrograph showing a Dohle body (arrows) consists of an aggregate of endoplasmic reticulum (X8280) (From McCall et al <sup>196</sup> courtesy of the authors and the Journal of Experimental Medicine)

Table 41-3. Several Methods Used to Measure Marrow Granulocyte Reserves

Agent	Dose and Route of Administration <sup>a</sup>	Peak Change in Neutrophil Concentration	Normal Response $\times 10^9/l$	Reference
<i>Endotoxin</i>				
Typhoid vaccine	0.5 ml SC	?	> 1.3 neutrophils at 5 hours	unpublished
Lipexal	0.1 $\mu$ g IV	3-5 hours	> 2.2 neutrophils*	183
Piromen	8 $\mu$ g IV	3-5 hours	> 2.4 neutrophils†	38
Etiocannabinolone	0.1 mg/kg IM	14-18 hours	> 2.6 granulocytes‡	192
<i>Adrenal Steroids</i>				
Prednisone	40 mg p.o.	5 hours	> 2.0 neutrophils*	173
Hydrocortisol	200 mg IV	3-4 hours	> 5.1 neutrophils‡	38

\*Polymorphonuclear neutrophils

†Total neutrophils

‡Granulocytes include band cells, segmented cells, eosinophils, and basophils

<sup>a</sup>SC, subcutaneously; IM, intramuscularly; IV, intravenously; p.o., orally

It has been suggested that alterations in LAP may be due to loss or inactivation of a regulator (modifier) rather than of the structural gene for LAP.<sup>137</sup> The induction of LAP formation in CML leukocytes in diffusion chambers implanted in mice is compatible with this thesis.<sup>168</sup>

Electrophoretic differences in the LAP of normal and chronic myelocytic leukemic leukocytes have been reported.<sup>217</sup>

#### Evaluation of the Functional Status of the Neutrophil System

With the widespread use of potent marrow-suppressing drugs of all kinds, in particular those used in the treatment of malignant disease, it has become more important than ever before to be able to assess the functional capacity of the neutrophil system. The measurement of blood neutrophil concentration and the proportions of the different types of cells, as well as the estimation of bone marrow cellularity and the myeloid:erythroid ratio, provide relatively crude estimates of the functional status of the neutrophil system; these measurements also are subject to large sampling errors.<sup>38,183</sup> Furthermore, it has been observed that, except in patients receiving steroid therapy<sup>158</sup> or in patients showing

defects in bactericidal capacity, as for example in some patients with Felty's syndrome,<sup>122,135</sup> little difficulty with infection is observed until concentrations of less than  $0.5 \times 10^9$  neutrophils/l occur.<sup>160</sup> Even at still lower levels, little or no difficulty may be encountered if other defense mechanisms are intact or if the patient can mobilize a few neutrophils at sites of inflammation.<sup>174</sup> For these reasons, several techniques have been described as a means for measuring marrow granulocyte reserves (MGR) with greater accuracy than that provided by crude estimates. These involve the injection of agents such as bacterial endotoxin or adrenal and other steroids that produce blood neutrophilia (Table 41-3). A typical normal response is shown in Figure 41-5; it can be seen that endotoxin and hydrocortisol both produce neutrophilia, but the magnitude and timing of the responses are somewhat different. Furthermore, the effect on leukocytes other than neutrophils differs with each compound. Etiocannabinolone produces neutrophilia, but does not affect other leukocytes.<sup>192</sup> The neutrophil response to the test agents is reduced in patients who have received chemotherapy or extensive x-ray therapy, or in whom the marrow is infiltrated by leukemia, myeloma cells, or other abnormal cell popula-

tions.<sup>38,183,192</sup> In some of these situations the MGR may be reduced even in the presence of normal blood leukocyte and differential counts. Detection of a decreased MGR is useful in predicting an increased risk of severe drug toxicity in cancer patients.<sup>183,185,192</sup>

Methods for assessing the ability of leukocytes to migrate *in vivo* into areas of induced inflammation such as skin windows,<sup>15</sup> skin blisters,<sup>2</sup> or plastic chambers placed over skin abrasions<sup>16</sup> also have been described. A decrease in cell migration into such lesions occurs in patients receiving prednisone<sup>15,163</sup> and may partially explain the increased susceptibility of these patients to infection. Abnormal migration is also seen in some diabetics, in patients in shock,<sup>167</sup> in those with leukemia,<sup>39</sup> those having a variety of leukopenic states, and when ethanol is ingested.<sup>167</sup> Young myeloid forms are less able to migrate into the induced lesions than are more mature forms.<sup>39</sup>

In addition to the above *in vivo* evaluations of the neutrophil system, a variety of *in vitro* methods have been devised to assess the ability of neutrophils to respond to chemotactic stimuli<sup>106</sup> and to phagocytize bacteria, fungi, and other particles,<sup>223a</sup> or kill them once they are ingested. The micropore diffusion chamber system<sup>165</sup> can be used to evaluate the ability of neutrophils to respond to known chemotactic stimuli. Cellular defects in response to chemotactic stimuli have been described in patients with adult rheumatoid arthritis, diabetes mellitus, disseminated lupus erythematosus, polymyositis,<sup>156</sup> Chediak-Higashi syndrome (see Chapter 42), and the "lazy leukocyte" syndrome.<sup>206</sup> Serum inhibitors<sup>227</sup> or deficiency of serum factors (complement and others)<sup>152,205,230</sup> have been described. Such defects apparently are common in cirrhotic patients.<sup>177</sup>

Phagocytosis has been measured by incubating cells with particles under a variety of conditions; the proportion of cells ingesting organisms, the mean number ingested per cell,<sup>166</sup> and the total number of particles ingested can be measured. Also the disappearance of viable bacteria or latex particles

from the medium or the uptake of radioactive bacteria by cells can be measured.<sup>179,200</sup> To date no clear defect in the cellular component of phagocytosis has been identified, perhaps because so serious a defect might be lethal, but decreased phagocytosis due to impaired opsonization of particles occurs in acquired hypogammaglobulinemia<sup>212</sup> and perhaps also in hereditary agammaglobulinemia and complement deficiency states.<sup>152,202</sup> Decreased phagocytic activity was reported in neonates and was thought to be related to decreased opsonization and to cellular factors,<sup>204</sup> but other workers have found phagocytic activity to be normal in neonates although they described a transient bactericidal defect that disappeared after 12 to 24 hours.<sup>171</sup> Still other studies have demonstrated increased metabolic activity of leukocytes in the newborn.<sup>212</sup> These discrepancies may be more apparent than real or may reflect the use of different techniques; their resolution awaits further study.

Leukocyte function can also be evaluated by measuring one or more of the metabolic events that are associated with phagocytosis and bacterial killing<sup>222</sup> or by determining the presence of viable intracellular organisms.<sup>172</sup> Defects in bactericidal function have been studied primarily in hereditary and familial states (see Chapter 42). However, decreased bactericidal activity is reported in severely burned patients and appears to be related to decreased content of lysosomal enzymes<sup>151</sup>; phagocytosis and degranulation were normal. The possibility of defects in bactericidal capacity has not yet been explored extensively, but none have been reported in alcoholics.<sup>177</sup> Since a number of pharmacologic and other agents are known to affect leukocyte metabolic and microbicidal functions (eg, vitamin A, steroid hormones, phenylbutazone, and colchicine<sup>193</sup>), such studies will have to be carefully controlled.

## The Eosinophil Series

Eosinophilia refers to an increase in the number of eosinophilic leukocytes above normal ( $>0.7 \times 10^9/l$ ) if calculated from the

total leukocyte and differential count (see Table 6-4, page 242) and  $>$  about  $0.2 \times 10^9/l$  if determined by absolute counting methods<sup>305</sup> (Chapter 6, page 263).

During infections in which neutrophilia occurs, eosinophils are generally reduced in number or disappear entirely, and, in animals with parasite-induced eosinophilia, infection leads to rapid disappearance of blood eosinophils.<sup>308</sup> The sudden disappearance of eosinophils has been attributed, without conclusive proof, to adrenal corticosteroid activity. Recovery from the infection is often heralded by a return of blood eosinophils.

#### Causes of Eosinophilia (Table 41-4)

**ALLERGIC DISORDERS.** In allergic disorders, usually the eosinophilia is moderate ( $4$  to  $11\%$ , total count  $0.2$  to  $1.5 \times 10^9$  cells/l), but in some instances it may be much higher, eg, in bronchial asthma,  $55.0 \times 10^9$  leukocytes/l with  $62.5\%$  eosinophils<sup>297</sup>; in angioneurotic edema, leukocyte counts as high as  $44.0 \times 10^9/l$  with  $27$  to  $85\%$  eosinophils.<sup>307</sup> Eosinophils are usually abundant in nasal discharges,<sup>271</sup> sputum, and skin wheals<sup>298,299</sup> of allergic individuals. In a patient with fatal bronchial asthma a large preponderance of eosinophils was found in the bone marrow and massive infiltrations were present in the myocardium.<sup>267</sup> Smoking has been reported to cause eosinophilia.<sup>326</sup>

Eosinophilia due to nitrofurantoin, par- amino salicylic acid, sulfonamides, and several other drugs<sup>279,283</sup> has been reported. Marked eosinophilia was observed in a patient with iodide sensitivity.<sup>290</sup> It is also noted after gold therapy for rheumatoid arthritis.<sup>315</sup>

**SKIN DISEASES.** The highest and most constant eosinophilia ( $10$  to  $60\%$ , sometimes with leukocytosis) has been observed in pemphigus and in dermatitis herpetiformis. Eosinophilia has been found in association with exfoliative dermatitis, psoriasis, pruritus, prurigo, eczema, dermatitis venenata, ichthyosis, mycosis fungoides, pityriasis rubra, facial granulomas,<sup>268</sup> and scabies. Its develop-

Table 41-4. Causes of Eosinophilia

- 1 Allergic disorders bronchial asthma urticaria, angioneurotic edema, hay fever, some instances of drug sensitivity
- 2 Skin diseases especially pemphigus and dermatitis herpetiformis
- 3 Parasitic infestations especially parasites that invade the tissues, eg. trichinosis, echinococcus disease, less regularly in intestinal parasitism
- 4 Loeffler's syndrome
- 5 Pulmonary infiltration with eosinophils ("PIE syndrome")
- 6 Tropical eosinophilia
- 7 Certain infections, eg. scarlet fever, chorea, erythema multiforme
- 8 Certain diseases of the hematopoietic system chronic myelocytic leukemia polycythemia vera, pernicious anemia, Hodgkin's disease, following splenectomy
- 9 Malignant disease of any type, especially with metastases or necrosis
- 10 Following irradiation
- 11 Miscellaneous disorders perianteritis nodosa, rheumatoid arthritis sarcoidosis, certain poisons, etc
- 12 As an inherited anomaly
- 13 Idiopathic

ment in such cases may depend on whether or not there is an allergic component; the degree of eosinophilia often appears to vary with the extent of involvement.

**PARASITIC INFECTIONS.** Parasites that invade tissues cause the most pronounced eosinophilia. A striking example of this is infection with *Trichinella spiralis*. Eosinophilia appears one to two weeks after ingestion of infected food and reaches a peak level at the end of the third week. Fluctuating eosinophilia then persists for six months or more and in some instances for years. Values as high as  $85\%$  with absolute eosinophil levels of  $15.0 \times 10^9/l$  have been found. It is noteworthy, however, that eosinophilia may be absent throughout the infection, and in severe fatal disease it is generally absent.<sup>318</sup> Studies of experimental trichiniasis in rats have shown that the eosinophilic response requires the presence of intact parasites in sufficient numbers and distribution to produce a wide-

spread intense inflammatory reaction (the injection of homogenized larvae intravenously or intact larvae subcutaneously or intramuscularly did not produce eosinophilia). It has also been found that thymus-derived lymphocytes play an essential role as mediators of the eosinophilia.<sup>252</sup>

In *echinococcosis*, eosinophilia is usually mild but occasionally it is pronounced. It is most likely to occur when slow leakage of cyst fluid into the tissues is occurring.<sup>292</sup> When suppuration occurs, eosinophilia disappears and neutrophilia develops. In amebic abscess of the liver, eosinophilia is not found, neutrophilia being common.<sup>300</sup> In the early stages of *cysticercosis*, moderate eosinophilia occurs, but this disappears when encystment takes place.<sup>275</sup> In *coccidioidal granuloma*, eosinophilia is sometimes found,<sup>321</sup> and it has been observed in human *toxoplasmosis*<sup>270</sup> and in human *Toxocara canis* infections.<sup>256, 323</sup> In schistosomiasis (*S. mansoni*), eosinophilia may be marked during the incubation period.<sup>325</sup> The liver fluke, *Clonorchis sinensis*, causes marked eosinophilia.<sup>259</sup> Eosinophilia was observed in only a third of patients with filariasis<sup>263</sup> and has not been found in those with trypanosomiasis or with kala azar. From 50 to 85% eosinophils may be seen in the blood of persons with *gnathostomiasis*<sup>272</sup> and 78% was reported in *Capillaria hepatica* infection.<sup>270</sup>

In malaria the presence and degree of eosinophilia is variable. In one series of 100 patients who were convalescing from malarial attacks the absolute eosinophil count ranged from 0 to  $1.35 \times 10^9/l$ . Eosinophilia decreased as time progressed but lasted for at least eight weeks. A pronounced fall (about 50%) was observed 24 to 36 hours before the onset of malarial symptoms. After the febrile period, in patients given treatment, the eosinophilia regained its previous level in about 10 days.<sup>303</sup>

Intestinal parasitism is less regularly associated with eosinophilia than is parasitism in which tissue invasion is prominent, and the eosinophilia is never as marked. Eosinophilia of 10 to 30 and even 69%<sup>250</sup> may occur in infection with *Uncinaria*<sup>290</sup> or *Strongyloides*

during the early stage, in *Ascaris* infection,<sup>255</sup> and in *Taenia* infections during periods of diarrhea and abdominal pain. In hookworm infection, eosinophilia, present in all subjects parasitized, was greatest in those with a worm burden greater than 100, but there was no good correlation between the eosinophil percentage and the worm burden.<sup>293</sup> Infestations with *Enterobius vermicularis* (*Oxyuris*), *Trichocephalus trichiuris*,<sup>314</sup> various amebae,<sup>291</sup> and the flagellates are usually not associated with eosinophilia.

**LOEFFLER'S SYNDROME.** In Loeffler's syndrome, a mild illness, characterized by transient pulmonary signs and infiltrates and presumably due to a hypersensitivity reaction, eosinophilia is characteristic.<sup>277</sup> A clinical and blood picture identical to that described by Loeffler has been observed in patients with cutaneous helminthiasis or "creeping eruption" caused by *Ancylostoma brasiliense*<sup>332</sup>; in infection with *Clonorchiasis*,<sup>200</sup> with *Ascariasis*,<sup>255, 316</sup> with *trichinosis*, and with amebae; and in tuberculosis, brucellosis, pollinosis, and coccidiomycosis.<sup>231</sup> A similar and perhaps identical disorder in children that is characterized by asthmatic symptoms, pulmonary infiltrates, joint symptoms, urticaria, convulsions, hepatomegaly, eosinophilia, and hyperglobulinemia appears to be due to invasion by a variety of nematode larvae (*visceral larva migrans*).<sup>256</sup>

**"PIE SYNDROME."** A distinction has been made between the "PIE syndrome" (pulmonary infiltration with eosinophilia) and Loeffler's syndrome.<sup>283, 301</sup> Loeffler's syndrome has been considered an acute, self-limited condition with a benign outcome, while PIE syndrome is a more chronic, relapsing illness often accompanied by cough, dyspnea, fever, sweats, malaise, and other manifestations. It is characterized by pleomorphic bilateral pulmonary infiltrations and marked eosinophilia. A few of the patients have had pericardial involvement.<sup>283</sup> It should be noted that the distinction is a quantitative one and may not be real.

The PIE syndrome may be produced by various infections (tuberculosis, coccidiomy-



cosis, brucellosis, viral or bacterial pneumonia, bronchiectasis) or may accompany the numerous parasitic infections causing eosinophilia mentioned above.<sup>283</sup> It may also be a manifestation of neoplastic conditions (Hodgkin's disease, eosinophilic granuloma), allergic reactions including those due to a variety of drugs,<sup>283</sup> collagen disorders, and a few miscellaneous diseases. Adrenocorticosteroid therapy has been very effective in therapy when not contraindicated by the underlying disease.

**TROPICAL EOSINOPHILIA.** Under the title of "tropical eosinophilia," a condition of unknown cause was described in India and South-East Asia.<sup>276</sup> The syndrome begins gradually with malaise; weight loss; low-grade fever; a paroxysmal, dry, hacking cough; and physical findings similar to those of bronchial asthma. Leukocytosis and severe eosinophilia are present. The spleen may be palpable. Roentgenograms reveal diffuse bilateral mottling of the lung fields resembling the picture of acute miliary tuberculosis. After several weeks a chronic subfebrile state is reached in which coughing paroxysms and wheezing may persist but weight loss usually ceases. At this stage, increased bronchial markings like those seen in chronic bronchitis are noted in the roentgeograms.

Symptoms may persist for a long time unless arsenotherapy or diethylcarbamazine<sup>273</sup> is given, to which the response is striking. Tropical eosinophilia, like Loeffler's syndrome, probably represents a hyper-immune reaction and is attributed to a variety of parasites, mainly microfilariae.<sup>276</sup>

**OTHER INFECTIONS.** Moderate eosinophilia may occur in association with various infections. Most noteworthy is scarlet fever. In the early stage of this disease, eosinophilia of 5 to 8% and even 17% may be found, especially when constitutional symptoms are mild and the rash is slight.<sup>280</sup> In chorea, eosinophilia is common and may reach values as high as 26%.<sup>280</sup> It is of interest that eosinophilia occurs also in erythema multiforme but not in acute rheumatic fever in which erythema is not a complication.<sup>320</sup> It has been stated that

eosinophilia occurs in gonorrhea when the posterior urethra, epididymis, or prostate is involved. It has been reported in leprosy.<sup>293</sup>

**DISEASES OF THE HEMATOPOIETIC SYSTEM.** In chronic myelocytic leukemia an increase in all types of granulocytes usually occurs, and even when the proportion of eosinophils is not increased, the absolute number is high. In polycythemia vera eosinophilia is not unusual. In about 20% of patients with Hodgkin's disease a slight or moderate eosinophilia occurs. In rare instances, values as high as 90% have been recorded.<sup>327</sup> Following splenectomy that has been performed for any cause, mild eosinophilia together with lymphocytosis may replace the neutrophilia that first appears. This may persist for several months.

A few patients with marked eosinophilia, leukocytosis, and hepatosplenomegaly have been described in whom the clinical and postmortem pictures suggested the diagnosis of eosinophilic leukemia.<sup>288,298,320</sup> Pulmonary infiltration and myocardial and/or pericardial involvement have been present in many of these subjects; the differentiation of their illness from that due to the PIE syndrome and collagen vascular disease<sup>317</sup> has been difficult. Some would distinguish patients having eosinophilia in which most of the eosinophils were mature and who experienced long survival ("hypereosinophilic syndrome"<sup>285,319</sup>) from patients with increased numbers of blasts in the blood and marrow who survived only a few months. Symptoms in the latter group more closely approximate those due to leukemia<sup>258,320</sup> (page 1475) than do those in the former.

In pernicious anemia, eosinophilia up to 20 or even 60% may develop. This was particularly common following the use of uncooked liver for treatment. In sickle cell anemia, eosinophilia is found occasionally.

**MALIGNANT DISEASE.** Eosinophilia is noted in malignant disease of various types.<sup>289</sup> In over 2,000 patients with malignant tumors of all types, 0.5% exhibited eosinophilia.<sup>289</sup> The eosinophilia was thought to be associated with dissemination or tumor necrosis.<sup>289,312</sup>

In rats with transplanted lymphomas and associated eosinophilia, the eosinophilia was not due to increased cell production; rather, it seemed to result from prolonged eosinophil survival in the blood, possibly caused by blockage of normal emigration pathways.<sup>328</sup>

**IRRADIATION.** Eosinophilia has been reported in radiological workers and in patients following courses of irradiation over trunk areas.<sup>276a</sup>

**MISCELLANEOUS DISORDERS.** Eosinophilia has been noted in a miscellaneous group of disorders: *perianteritis nodosa* (in about 18% of subjects, with values as high as 84%)<sup>310</sup>; *rheumatoid arthritis* complicated by vasculitis and pleuritis<sup>312</sup>; extensive caseous tuberculosis of the lymph nodes<sup>305</sup>; ulcerative colitis<sup>322</sup>; and congenital cardiovascular malformation.<sup>306</sup> It has occurred as a benign, idiopathic condition in which leukocyte counts as high as  $138.0 \times 10^9$  cells/l were noted, 93% of the cells being eosinophils.<sup>287</sup> Allegedly it has followed poisoning by copper sulfate, by camphor, by pilocarpine, and by phosphorus.

**HEREDITARY EOSINOPHILIA.** Mild eosinophilia not associated with any of the conditions described above has been reported in about 19 families.<sup>292-313</sup> The condition is inherited as an autosomal dominant trait, is benign, and must be distinguished from eosinophilia of other causes.

**IDIOPATHIC EOSINOPHILIA.** In some patients with eosinophilia no cause can be found even after extensive evaluation.

## The Basophil Series<sup>345</sup>

Basophilic leukocytosis or basophilia refers to an increase in the number of basophilic leukocytes above normal (that is,  $>0.15 \times 10^9$  cells/l if calculated from the total leukocyte and differential count [Table 6-4, page 242] and  $>0.1 \times 10^9$  cells/l if counted directly) (page 266). The basophil concentration is increased in myxedema and is low in hyperthyroidism, during ovulation, in pregnancy,<sup>352</sup> and under conditions of

stress.<sup>347</sup> Basophil numbers may also be increased in ulcerative colitis,<sup>347</sup> chronic sinusitis, smallpox, chickenpox, following the injection of foreign protein,<sup>345</sup> and in some cases of nephrosis.<sup>241</sup> They are frequently increased in chronic myelocytic leukemia, polycythemia vera, myeloid metaplasia, following splenectomy, in some chronic hemolytic anemias, and in Hodgkin's disease.

Infiltration of basophils has been demonstrated in areas of contact dermatitis, apparently in response to an initial interaction between antigen and lymphocytes. Thus the basophil appears to be involved in certain types of delayed hypersensitivity,<sup>343</sup> and IgE appears to be an essential intermediate in the reaction.<sup>344</sup> Blood basophilia does not appear to accompany such local reactions. Estrogens and antithyroid drugs produce increased values,<sup>352</sup> while adrenal corticosteroids and ACTH produce a decrease<sup>346</sup> as do acute infection<sup>349</sup> and x-irradiation or chemotherapy.<sup>349</sup>

In *urticaria pigmentosa* and in *systemic mast cell disease*,<sup>350</sup> blood basophils are not usually or greatly increased, but mast cells may be increased in the bone marrow and they may also be seen in the blood (Chapter 49). The presence of progressive anemia, leukopenia, thrombocytopenia, and hepatosplenomegaly may suggest leukemia (page 1502), but a pigmented maculopapular rash and dermatographism are characteristic. There often is widespread skeletal disease and there may be a marked increase in serum histamine levels and decreased serum serotonin and hyaluronic acid; in the urine, chondroitin sulfuric acid B is found.<sup>340</sup>

## The Monocyte Macrophage Series

Monocytosis refers to an increase in blood monocytes above the upper limit of normal ( $>0.95 \times 10^9$  cells/l) (see Table 6-4).

### Causes of Monocytosis (Table 41-5)

**BACTERIAL INFECTIONS.** Monocytosis may be present in certain bacterial infections, in-

cluding actively progressing tuberculosis, subacute bacterial endocarditis, septicemia, syphilis, and brucellosis.<sup>368,367</sup> Infection by *L. monocytogenes* produces a marked mononuclear response (apparently both monocytic and lymphocytic<sup>368</sup>) in rabbits<sup>376</sup> and other animals, but rarely does so in man.<sup>370</sup>

The role of the monocyte in tuberculosis has been studied intensively.<sup>363,366</sup> It was shown that this cell plays an important part in the cellular reaction to the tubercle bacillus. The phospholipids of this organism are partially degraded within monocytes and macrophages and cause the transformation of these cells to epithelioid cells. The monocyte is thus the chief cell in new tubercle formation. This activity may be reflected in the blood, monocytosis being regarded as evidence of active extension of the tuberculous process.<sup>360,363</sup> In following tuberculous infection in animals or man the *monocyte: lymphocyte ratio* has proved useful.<sup>360,363,374</sup> The normal ratio is about 0.3, or less; in active tuberculosis the number of blood monocytes may increase to or exceed the number of lymphocytes. A ratio of 0.8 to 1.0 or higher indicates active exudation and an unfavorable prognosis. With healing the number of monocytes decreases, while the lymphocytes may increase, resulting ultimately in a return to the normal ratio.

In endocarditis caused by *Streptococcus viridans*, monocytosis may occur, as many as one third of the leukocytes being of this type.<sup>377</sup> Monocytosis may occur in the absence of leukocytosis and its magnitude is subject to great fluctuations.<sup>372</sup> Histiocytic cells (sometimes referred to as reticuloendothelial cells, macrophages, or transformed monocytes) (see Chapter 6, page 267) may be found in the blood as well. They occur in about 25% of patients with subacute bacterial endocarditis.<sup>369</sup> They may exhibit vacuoles (perhaps phagosomes) in their cytoplasm and have been seen to contain engulfed erythrocytes, neutrophils, or lymphocytes.<sup>367</sup> Histiocytes are more abundant in the first drop of blood obtained from the ear lobe than from the finger<sup>364</sup> and their presence should suggest endocarditis even in the absence of

Table 41-5. Causes of Monocytosis

- 1 *Certain bacterial infections* tuberculosis, subacute bacterial endocarditis, syphilis, brucellosis
- 2 *During recovery from acute infection* and from agranulocytosis, in "leukopenic infectious monocytosis"
- 3 *Many protozoal and rickettsial infections* malaria, typhus, Rocky Mountain spotted fever, trypanosomiasis, kala azar, Oriental sore
- 4 *Lymphoma, leukemia and other hematologic disorders* monocytic leukemia, Hodgkin's disease and other lymphomas, chronic myelocytic leukemia and "myeloproliferative" disorders, multiple myeloma, lipid storage diseases
- 5 *Malignancy* carcinoma of ovary, stomach, breast
- 6 *Collagen vascular disease* lupus erythematosus, rheumatoid arthritis
- 7 *Granulomatous disease* sarcoidosis, ulcerative colitis, regional enteritis
- 8 *Tetrachloroethane poisoning*

positive blood cultures.<sup>369</sup> An occasional histiocyte may be found in the blood in a variety of other disorders.<sup>365</sup>

**RECOVERY FROM ACUTE INFECTION.** Transient monocytosis may develop in patients recovering from acute infection; if complications such as empyema occur the monocytosis may persist.<sup>366</sup> Monocytosis also occurs in the recovery phase of agranulocytosis (page 1298).

Increased numbers of monocytes and mononuclear leukocytes have been reported in infants and young children with probable viral disease,<sup>380</sup> and in adults with *collagen vascular disease*, arthritis, and other inflammatory processes.<sup>371</sup> In the latter patients, the mononuclear cells exhibited enhanced ability to incorporate tritiated thymidine in their DNA, but it is not clear whether the cells were atypical lymphocytes (presumably participating in an immune response to their disease), monocyte precursors released in response to tissue injury, or both.

*Leukopenic infectious monocytosis* is the name proposed for a rare acute illness that often has been associated with necrotizing ulcerations of the mucous membranes and has been accompanied by normal or decreased leukocyte count, neutropenia, and monocyto-

sis.<sup>378,379</sup> The prognosis has been favorable in most cases, but deaths have occurred.<sup>378</sup> In many instances a history of drug ingestion was elicited, thus suggesting that this condition may be a variant of agranulocytosis. In some instances, however, drugs could not be clearly incriminated.<sup>379</sup>

**PROTOZOAL AND RICKETTSIAL INFECTIONS.** Monocytosis occurs in many protozoal and rickettsial infections such as *malaria*, *typhus fever*, and *Rocky Mountain spotted fever*. In kala-azar as many as 45% monocytes may be seen, but because of the leukopenia an absolute monocytosis is unusual.<sup>386</sup> In Oriental sore, trypanosomiasis, and in some patients with syphilis,<sup>381</sup> especially those having dementia paralytica,<sup>385</sup> monocytosis occurs.

**LYMPHOMAS AND HEMATOLOGIC DISORDERS.** Monocytosis is found in lymphomas and other hematologic disorders. The highest blood monocyte concentrations are seen in *monocytic leukemia*, but values greater than  $1.0 \times 10^9$  cells/l are found in about one third of patients with *Hodgkin's disease*,<sup>373</sup> and absolute monocytosis may also be seen in *reticulum cell (histiocytic) and other lymphomas*. Monocytosis may occur in *Gaucher's disease*,<sup>381</sup> *Niemann-Pick disease*, *Hand-Schüller-Christian disease*, *subacute and chronic myelocytic leukemia*, in other "myeloproliferative disorders," and in *multiple myeloma*.<sup>373</sup>

**MALIGNANT DISEASE.** In malignant neoplasms, such as carcinoma of the ovary, stomach, or breast, monocytosis is not uncommon<sup>373</sup> and may reflect some resistance to the tumor.

**COLLAGEN VASCULAR DISEASE.** Absolute monocytosis is present in over 50% of patients with collagen vascular disease.<sup>373</sup>

**GRANULOMATOUS DISEASES.** Monocytosis may be associated with granulomatous diseases such as *sarcoidosis*, and with *ulcerative colitis* and *regional enteritis*. It has been reported in patients with fever of unknown origin and in patients in whom no clear diagnosis could be established.<sup>373</sup>

**TETRACHLORETHANE POISONING.** Monocytosis has occurred in tetrachlorethane poisoning.<sup>375</sup>

## The Lymphocyte Series

Lymphocytosis refers to an increase in blood lymphocyte concentration above the normal level of  $4.0 \times 10^9$  cells/l (see Table 6-4), while lymphopenia refers to a decrease below  $1.5 \times 10^9$  cells/l. As already discussed (Chapter 2 and Fig. 2-11, page 58) the blood of infants and young children contains a higher proportion and concentration of lymphocytes than that of adults and these values gradually decrease toward adult values as maturation progresses.

### Causes of Lymphocytosis

**CERTAIN ACUTE INFECTIONS.** Lymphocytosis (Table 41-6) is rare during *acute bacterial infections* except in *pertussis*. In 63% of children with pertussis who were over six months old a lymphocyte concentration greater than  $11.0 \times 10^9$  cells/l was observed during the first 2 weeks of infection; during the third week, 81% had lymphocytosis, after which this finding was less common. Lymphocytosis was seen in only 20 to 25% of children with pertussis who were younger than six months of age.<sup>410</sup> Usually the total leukocyte count in pertussis is about  $20.0 \times 10^9$ /l and the lymphocytes constitute 60% or more of this number<sup>421</sup>; however, the lymphocytosis may exceed  $50 \times 10^9$  cells/l in 4 to 21% of the subjects,<sup>410,711</sup> these high values usually

Table 41-6. Causes of Lymphocytosis

1. Certain acute infections: pertussis, acute infectious lymphocytosis, infectious mononucleosis, infectious hepatitis
2. Certain chronic infections: brucellosis, tuberculosis, secondary and congenital syphilis
3. Hematopoietic disorders: lymphocytic leukemia, chronic and acute, some cases of lymphosarcoma, heavy chain disease
4. Relative lymphocytosis may be seen in exanthems, after the initial stage, especially in mumps and German measles, during the stage of convalescence from acute infections, in thyrotoxicosis, in most conditions associated with neutropenia

being associated with complications (see Experimental Production of Leukemoid Reactions, page 1303). The lymphocytes are of the small, mature type.

**Acute infectious lymphocytosis** is a benign disease having an incubation period of 12 to 21 days. It is characterized by lymphocytosis and is seen most frequently in children although it has been reported in young adults as well. The onset is marked by varying degrees of constitutional reaction. No symptoms whatever were observed in most of the patients, whereas, in one series,<sup>392</sup> fever occurred in 30% and gastrointestinal symptoms such as diarrhea (5%) and vomiting (rare) were experienced by a few of the subjects. Occasionally abdominal symptoms and signs severe enough to suggest an acute surgical condition have been observed.<sup>392,417,422</sup> In other subjects, upper respiratory symptoms, signs of central nervous system involvement, or skin rash has been noted.<sup>418,422</sup> Lymphadenopathy and splenomegaly are usually absent. The lymphocytosis persists for three to seven weeks and consists of predominantly normal adult lymphocytes; the average peak value is  $34.0 \times 10^9/l$ ,<sup>392</sup> but values as high as  $117 \times 10^9$  cells/l have been reported.<sup>417</sup> Because eosinophilia of marked degree (average  $2.3 \times 10^9/l$ ) has been observed in many of the patients, the possibility of parasitism as the cause of this condition has been entertained.<sup>392,407</sup> No anemia or thrombocytopenia has been observed. The bone marrow shows some increase in lymphocytes (30 to 40%). Lymph node biopsy in two instances revealed nondiagnostic degeneration of the follicles and proliferation of the sinus reticuloendothelium.<sup>422</sup> The heterophil agglutination test has given uniformly negative results. An increase in spinal fluid lymphocytes has been reported in occasional subjects. The disease appears to be infectious and moderately contagious, but as yet no definite etiologic agent has been clearly incriminated. Both an adenovirus<sup>413</sup> and a Coxsackie A virus<sup>407</sup> have been suggested as etiologic agents. EB virus and cytomegalovirus do not appear to be involved.<sup>395</sup>

Lymphocytosis is characteristic of *infec-*

*tious mononucleosis*, but, in contrast to pertussis and infectious lymphocytosis, many atypical and large lymphocytes are present in the blood (see Chapter 43). Lymphocytosis with many atypical cells is noted also in the *post-transfusion syndrome* (probably cytomegalovirus disease, see Chapter 43) and may be found in infectious hepatitis as well.<sup>411</sup> As already mentioned, atypical lymphocytes and monocytes may sometimes be difficult to differentiate and the numbers of both may be moderately increased in a variety of disease states (see page 1287).

**CHRONIC INFECTIONS.** Occasionally, absolute lymphocytosis is found in association with certain chronic infections such as *brucellosis*<sup>398</sup> and with healing tuberculosis (page 1287). In the secondary stage of syphilis, lymphocytosis may occur and in *congenital syphilis* leukocyte counts as high as  $60 \times 10^9$  cells/l, the cells consisting mainly of lymphocytes, have been reported.<sup>399</sup>

**HEMATOPOIETIC DISORDERS.** Lymphocytosis is characteristic of chronic lymphocytic leukemia (see Chapter 49) and is present in most patients with acute lymphoblastic leukemia (see Chapter 47). It may also be found in some patients with non-Hodgkin's lymphomas (Chapter 51) and lymphocyte-like cells are present in the blood of patients with heavy chain disease (Chapter 53). In all of these conditions, lymphoblasts and intermediate and atypical lymphocyte forms are present in the blood in variable numbers.

**RELATIVE LYMPHOCYTOSIS.** During convalescence from acute infections, lymphocytosis is said to be common,<sup>415</sup> but such lymphocytosis usually is relative rather than absolute. The same appears to apply to German measles<sup>406</sup> and to mumps, in which 44 to 68% of the leukocytes may be lymphocytes<sup>423</sup> but the associated leukopenia results in normal absolute concentrations. Relative lymphocytosis also occurs in most conditions associated with neutropenia.

In *thyrotoxicosis*, Kocher (1908) described leukopenia, due to a reduction mainly of granulocytes, with relative and absolute lym-

phocytosis and moderate eosinophilia. However, these changes are inconstant, slight in degree, and are not reliable indices of prognosis or response to therapy.<sup>400</sup> Lymphocytic infiltration of the thyroid gland is correlated with the degree of lymphocytosis, and hyperplasia of lymphoid organs such as lymph nodes and spleen may be associated.

From time to time, attention has been called to *morphologic variations in lymphocytes in disease*.<sup>427</sup> In response to stress, lymphocytes that are characterized by nuclear and cytoplasmic distortion and by more cytoplasm than is normally seen may appear in the blood of mice.<sup>401</sup> These cells are similar to the atypical cells seen in infectious hepatitis,<sup>111</sup> serum sickness, and chronic infection.<sup>401</sup> An increase in lymphocyte size has also been described in adrenal insufficiency and in hyperthyroidism, these changes disappearing after appropriate therapy.<sup>405</sup> *Binucleate lymphocytes* appear in the blood in increased numbers after small doses of irradiation. Normal values are less than 4 binucleated cells/10<sup>4</sup> lymphocytes. During the second week, after a single exposure to 20 rads, 4 to 13 binucleated cells/10<sup>4</sup> lymphs were seen, and, after 50 rads, 6 to 32 binucleated cells were observed.<sup>416</sup>

*Lymphocytopenia* is seen in most acute infections and illnesses including heart failure,<sup>408, 420</sup> pneumonia,<sup>420</sup> pancreatic necrosis,<sup>404</sup> active tuberculosis,<sup>420</sup> carcinoma of varied origin,<sup>420, 421, 425</sup> uremia,<sup>423</sup> the lymphomas<sup>423</sup> (especially Hodgkin's disease<sup>390, 411</sup>), lupus erythematosus and other collagen diseases,<sup>428</sup> agranulocytosis (page 1298), malaria,<sup>130</sup> some immunologic deficiency syndromes,<sup>414</sup> and in a number of patients with no apparent disease.<sup>428</sup> In heart failure,<sup>408</sup> and probably other acute illnesses, the lymphopenia appears to be due to elevated plasma cortisol levels; the mechanism in other conditions is not understood. In addition, a number of agents and experimental procedures produce lymphopenia. These include adrenal steroid administration (Fig. 41-5), x-irradiation,<sup>397, 398, 409</sup> some chemotherapeutic agents,<sup>393, 394</sup> antilymphocyte serum,<sup>402</sup> thoracic duct drainage,<sup>403</sup> and

neonatal thymectomy.<sup>390</sup> In experimental animals, moderate exposure to dry heat, sunlight, the short ultraviolet rays<sup>396</sup> (less than 30 nro), and roentgen rays<sup>412</sup> has, after an initial reduction in lymphocytes, been shown to cause a marked lymphocytosis that persists for several weeks.

## Plasma Cells

Plasma cells are rarely found in the blood and when present their significance is obscure. In rubella as many as 19% Türk and plasma cells have been observed,<sup>412</sup> while in scarlatina, measles, and chickenpox<sup>414</sup> these cells are less numerous.<sup>423</sup> Their number may be increased in serum reactions,<sup>393, 419</sup> multiple myeloma, benign lymphocytic meningitis,<sup>426</sup> skin diseases, and sometimes in infectious mononucleosis. In plasma cell leukemia, they are the predominating cells.

## Agranulocytosis and Drug-Induced Neutropenia

### History

In 1922, Werner Schultz<sup>462</sup> drew attention to a syndrome of unknown cause which he had observed especially in women of middle age, and which was characterized by severe sore throat, marked prostration, extreme reduction or even complete disappearance of the granulocytes from the blood, and, in rapid succession, sepsis and death. He considered this to be a clinical entity which he called agranulocytosis. A detailed report of a similar condition had been published by Brown<sup>454</sup> in 1902 and by Türk<sup>463</sup> in 1907. However, it was not until the description of six cases by Schultz that general interest in this disorder was aroused.

A great variety of names have been used in referring to the syndrome described by Schultz, eg, agranulocytic angina; idiopathic, malignant, or pernicious leukopenia; granulocytic hypoplasia. Here the term "agranulocytosis" will be used to refer to the acute symptom-complex described by Schultz,

ile the terms "neutropenia," "granulocytosis," and "leukopenia" are used to refer to changes in the blood.

### ology

Although the majority of cases of agranulocytosis have been reported in the United States and Europe, agranulocytosis has been observed in all parts of the world.<sup>550</sup> The disease makes its appearance everywhere in the form of isolated cases.

Relatively few cases were described between 1922 and 1929, but in the next five years many were reported.<sup>555</sup> In 1931, Acke<sup>457</sup> pointed out that the sudden appearance of cases of agranulocytosis corresponded with the introduction of certain 1-tar derivatives. The disorder was especially common in the countries in which such drugs were in great use, and those affected were chiefly women of good economic status, as well as nurses and other medical workers whom these drugs were easily accessible. Acke noted that a history of the use of one of the coal-tar derivatives was often obtained in cases of agranulocytosis. Shortly afterwards, several reports<sup>460,480,490</sup> appeared that incriminated aminopyrine (Pyramidon) as the offending drug. It was noted that the majority of patients who developed agranulocytosis were adults, usually past middle age.<sup>460</sup> In proportion to other causes, agranulocytosis was a relatively frequent cause of death among people over 40 years of age, whereas it was rare in those under 25 years of age. Satisfactory accounts of typical agranulocytosis were found in only nine children.<sup>460</sup> The incidence among females as compared with males was about 2 or 3 to 1.<sup>460</sup> In relation to aminopyrine, it was reported<sup>480</sup> that the mortality in six patients who continued the use of drugs containing aminopyrine was 100%, whereas in a group of eight patients who stopped taking aminopyrine, only two died. The increased incidence of agranulocytosis in Denmark until 1934 corresponded exactly with the increased use of aminopyrine.<sup>460</sup>

After 1934 the number of cases of agranu-

locytosis decreased. This coincided with the reduced sales of aminopyrine following reports of its injurious effects.<sup>460</sup> Later, however, as new drugs were introduced new cases appeared.<sup>477</sup> A pattern was repeated which has now become a familiar one. A new agent is described and in a few years it is widely used. At first it is hailed as being nontoxic; ultimately a report appears describing the development of agranulocytosis in association with its use. This is followed by another case, and another, and the circumstantial evidence becomes impressive. The drug loses popularity and is employed with more discrimination than before. The number of cases of agranulocytosis due to this agent decreases sharply, only to be replaced by other cases as new drugs are added to the therapeutic armamentarium.

With the sharp reduction in the use of aminopyrine and the consequent rarity of cases of agranulocytosis due to this cause, some physicians were led to assume that the potential harmful effects of this drug had been greatly exaggerated. The fallaciousness of this view became clear when an increasing number of cases of agranulocytosis, with a number of deaths, were recognized to be associated with the increasing popularity of dipyrone, a derivative of aminopyrine with the same pharmacologic effects and therapeutic indications.<sup>474</sup> Potentially harmful itself, this drug also was shown to produce granulocytopenia in a patient who had recovered from aminopyrine agranulocytosis.<sup>474</sup>

### Agents Occasionally Associated with the Development of Leukopenia and Neutropenia (Table 41-7)

A number of physical agents, chemicals, and drugs produce blood dyscrasias such as anemia (Chapters 14 and 56), thrombocytopenia (Chapter 34), neutropenia, and leukopenia or various combinations of these, including pancytopenia (Chapter 56). Leukopenia is probably the most common; 42% of the cases reported to the AMA registry of adverse drug reactions were of this type.<sup>450,461</sup> Leukopenia is due mainly to a

**Table 41-7. Agents Occasionally Associated with Granulocytopenia and Leukopenia**

**1 Analgesics and sedatives**

*aminopyrine*\* and compounds containing it (Pyramidon Aminopyrine<sup>474</sup> Allonal Amidophen Cibalgol Veramon Amytal Compound Corosedine Optalidon Somnosol Veropyron<sup>472</sup> antipyrine<sup>475</sup> Causalin<sup>470</sup> neonal compound Neurodyne Peralga Pyraminol Yeast vite etc.) (see Ref 477)

*dipyron*\* a sulfonated aminopyrine and compounds containing it (Novalgil Novaldin<sup>476</sup> Migesic Pyralgin etc.)<sup>478</sup>

phenacetin (Acetophenetidin)<sup>450</sup> 477 acetanilid<sup>477</sup> allylisopropyl barbituric acid (New Allonal)<sup>474</sup> other barbiturates<sup>450</sup> 453 482

*phenylbutazone*\* (Butazolidin)<sup>485</sup> 488 oxyphenbutazone,<sup>478</sup> 482 487 indomethacin<sup>450</sup> acetylsalicylic acid<sup>453</sup> carbamazepine (Tegretol)<sup>472a</sup>

**2 Phenothiazines<sup>504</sup> and other tranquilizers**

*chlorpromazine*<sup>509</sup> (Thorazine) mepazine<sup>509</sup> (Pacitol) promazine<sup>504</sup> (Sparine) thioridazine (Mellaril) prochlorperazine (Compazine)<sup>506</sup> (mpiamine (Tofranil)<sup>503</sup> Diazepam<sup>502a</sup> etc maprobamate (Miltown Equanil)<sup>552</sup>

**3 Sulfonamides (antibacterial)**

sulfanilamide<sup>477</sup> 520 524 sulfisoxazole<sup>524</sup> (Gantresin) sulfamethoxyypyridazine (Kynax) saficylazosulfapyridin<sup>521</sup> 522 524 (Azasulfidine) sulfapyridine<sup>521</sup> sulfathiazole<sup>524</sup> sulfediazine<sup>523</sup> succinyl sulfathiazole<sup>524</sup> sulphasazine<sup>521</sup> (Salazopyrin) sulfaquinoxaline<sup>520</sup>

**4 Sulfonamides (non antibacterial)**

chlorothiazide<sup>551</sup> 542 (Diuril) *carbutamide*,<sup>451</sup> tolbutamide<sup>450</sup> 458 (Orinase) chlorpropamide<sup>543</sup> (Diabinese) chlorothalidone<sup>540</sup> (Hygroton) acetazolamide<sup>545</sup> (Diamox)

**5 Antithyroid drugs**

thiouracil<sup>557</sup> 542 propylthiouracil<sup>551</sup> methimazole<sup>553</sup> (Tapazole) carbimazole<sup>450</sup> 552 540

**6 Anticonvulsants**

diphenylhydantoin sodium<sup>450</sup> (Dilantin) trimethyloxazolidine<sup>570</sup> 571 (Tridione) methyl phenylethyl hydantoin<sup>450</sup> 570 (Mesantoin) phethenylate<sup>572</sup>

**7 Antihistamines**

tripelennamine<sup>581</sup> (Pyribenzamine) methaphenilene<sup>582</sup> (Diatin) thenalidine<sup>580</sup> (Sandostene)

**8 Antimicrobial agents**

*chloramphenicol*<sup>456</sup> thiosemicarbazone<sup>592</sup> (Tibione) ristocetin<sup>593</sup> 402 (Spontin) methicillin<sup>598</sup> 401 (Staphicillin), ampicillin<sup>594</sup> novobion<sup>604</sup> organic arsenicals<sup>592</sup> 595 400 nitrofurantoin<sup>599</sup> (Furadantin) para amino benzoic acid<sup>603</sup> metronidazole<sup>596</sup> 408 (Flagyl) cephalothin<sup>597</sup> (Keflin) isonicotinic acid hydrazide (INH)<sup>599</sup>

**Table 41-7 (Continued)**

**9 Miscellaneous agents**

dinatriphenol,<sup>622</sup> phenindione,<sup>629</sup> 630 632 639 penicillamine<sup>620</sup> thioglycolic acid (cold wave),<sup>621</sup> mercurial diuretics<sup>625</sup> DDT,<sup>632</sup> 638 ethacrynic acid<sup>635</sup> procainamide<sup>634</sup> 637 634 diethazine,<sup>623</sup> cinchophen,<sup>631</sup> dapsone<sup>628</sup> antimony<sup>640</sup> (Neostibosan) pythyldione<sup>614</sup> (Presidone) quinine<sup>460</sup> 477 plasmochin,<sup>509</sup> gold salts<sup>622</sup> rauwolfia (Ajmalin)<sup>637</sup>

\*Drugs italicized are those most frequently associated with leukopenia

reduction in neutrophils, but, if severe, other leukocyte forms may be reduced as well. As discussed earlier (page 1272), some agents produce neutropenia and leukopenia in everyone to whom they are administered if given in sufficient dose (Table 41-2), and since they also frequently produce anemia and thrombocytopenia (ie, pancytopenia) they will be considered in Chapters 55 and 56. Other agents produce neutropenia unpredictably and in only a few of the patients exposed to them (Table 41-7). These will be discussed here.

**Pathogenetic Mechanisms**

Agents that are only occasionally associated with neutropenia presumably act via some inherent sensitivity to a drug or one of its metabolic products. Such drugs can be grouped in at least two categories, according to the changes that are usually observed and the possible mechanisms involved.

1. In some cases, usually after the patient has had intermittent and repeated exposure to a drug, an acute, apparently immunologically mediated, hypersensitivity reaction takes place with sudden disappearance of neutrophils from the blood (*abrupt-onset neutropenia*). For example, the agranulocytosis produced by aminopyrine is of this type as judged from the clinical observation that a small challenging dose given to patients who had recovered from an episode of agranulocytosis resulted in disappearance of all neutrophils from the blood in 6 to 10 hours<sup>453</sup> or less.<sup>459</sup> Additional observations of this



kind have been reported.<sup>450</sup> Transfusion of blood drawn from a sensitive patient three hours after receiving aminopyrine produced granulocytopenia in normal subjects within 40 minutes; the neutrophil concentration returned to normal in four hours. This patient's serum caused lysis of granulocytes in vitro in the presence of drug.<sup>456</sup> A second attack of agranulocytosis was produced by the intradermal injection of drug incubated with serum.<sup>455</sup> In another sensitive patient, leukocytotoxic and leukoagglutinating antibody (present in the IgG and IgM serum protein fractions) persisted in the blood for one year.<sup>481</sup>

Although, pathophysiologically, one would postulate that such acute developing neutropenia results from *rapid, immunologically mediated destruction* of neutrophils, the immunologic mechanisms are not clear. Lysis of granulocytes by the offending drugs has been difficult to reproduce in vitro and the diagnostic significance of the leukocyte agglutination test has been questioned.<sup>456,459</sup> It has been postulated that the offending drug alters the leukocytes or complexes with them as a haptene causing them to become antigenic and thereby inducing a cell specific antibody response. However, there is no clear evidence that the chemical combination of drug with granulocyte components leads to the cell no longer being recognized as "self."<sup>456</sup> Another possibility is that the drug (or a metabolite) serves as a haptene and combines with protein to form a complex antigen to which antibody is then formed. Soluble antigen-antibody complexes might then coat leukocytes and lead to their destruction by various means.<sup>456</sup> The finding that one such antibody reacted with either patient's or control leukocytes supports this hypothesis.<sup>481</sup> Reasoning by analogy with the immune hemolytic states, several types of immune cell damage might be expected to occur such as direct cell lysis, agglutination, or sequestration and destruction in the spleen or other sites, but these are as yet unsubstantiated.

2. In other patients, after several weeks of drug treatment, neutrophil concentration may

begin to decrease slowly and may reach such low levels that discontinuance of the drug is necessary (*slow or delayed onset neutropenia*). Not infrequently, however the neutrophil concentration may stabilize at a reduced level or may even return toward normal in the face of continued drug administration.<sup>554</sup> The granulocytopenia associated with phenothiazine administration, and perhaps also with thiouracil, hydantoins, and the sulfonamides, often develops in this way and is presumably due to *decreased cell production*. In the case of chlorpromazine, at least three weeks of drug therapy seem necessary before granulocytopenia develops, and the bone marrow is usually hypocellular at the time granulocytopenia appears. It has been shown in vitro that chlorpromazine inhibits nucleic acid synthesis by marrow cells both in sensitive subjects and in some patients not known to be sensitive to the drug.<sup>508</sup> Labeled DNA and RNA precursor uptake by marrow cells was shown to be reduced in susceptible patients.<sup>508,510,511</sup> A suggested explanation for the fact that neutropenia develops only in a minority of patients given chlorpromazine is that only subjects with a limited marrow proliferative potential are unable to overcome the delay in DNA synthesis produced by the drug and, as a consequence, develop granulocytopenia.<sup>510</sup>

It should not be concluded that the above mechanisms are mutually exclusive. Some drugs appear to produce either abrupt-onset neutropenia or slow-onset neutropenia, eg, sulfonamides or antithyroid drugs. The mode of onset, furthermore, may reflect prior sensitization that in turn reflects the more usual manner of administration of the drug in question, eg, intermittent use for analgesic purposes, as in the case of aminopyrine, or continuous use for longer periods, as is usually the case with sulfonamides or antithyroid drugs. Still other drugs may act by means other than immunologic ones, eg, as metabolic antagonists in susceptible individuals. The relative incidence of neutropenia apparently caused by drugs and reported to the AMA Council on Drugs is shown in Figure 41-7.

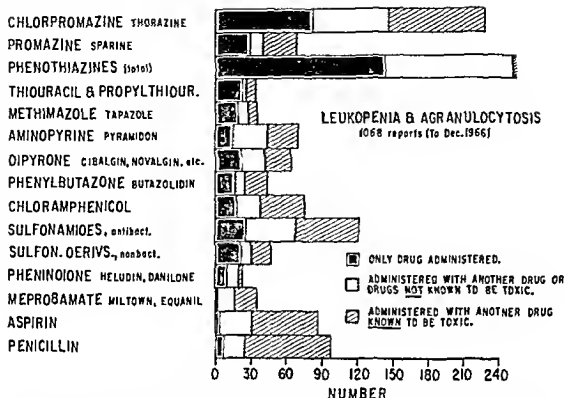


Fig. 41-7 Numbers of cases of leukopenia and agranulocytosis reported to Registry on Adverse Reactions, Council on Drugs, AMA grouped according to drug or type of compound (From Wintrobe,<sup>441</sup> courtesy of the author and *Journal of the Royal College of Physicians, London*)

### Analgesics and Sedatives

A wide variety of proprietary and prescription medications containing *aminopyrine* or slight chemical modifications of this drug have been marketed, often with names that disguise the contents (Table 41-7). All of these have been incriminated as causing agranulocytosis<sup>472,476,477</sup> and they account for perhaps 75% of reported cases.<sup>453</sup> The incidence of agranulocytosis due to *aminopyrine* is estimated to be about 1%.<sup>472,484</sup> although in some large series no cases were noted.<sup>472,486</sup>

Much less commonly, *phenacetin* (Acetophenetidin)<sup>450,477</sup> and *acetanilid*<sup>477</sup> have been incriminated. Although many of the preparations containing *aminopyrine* also contained barbiturates,<sup>490</sup> only rarely has good evidence been provided that *barbiturates* alone produce agranulocytosis.<sup>450,453,473,483</sup>

*Phenylbutazone* is closely related chemi-

cally to *aminopyrine*<sup>485</sup> and has caused rapidly developing<sup>450,453,483,488</sup> as well as slowly developing neutropenia.<sup>488</sup> The administration of *phenylbutazone* to patients known to be sensitive to *aminopyrine* did not produce neutropenia or agranulocytosis,<sup>485</sup> although cross reactivity has been demonstrated *in vitro*.<sup>491</sup> The neutropenia developing during the first four weeks of therapy with *phenylbutazone* is said to be preceded often by skin rash, whereas neutropenia developing after the fourth week is not.<sup>492</sup> No apparent relation between dosage and the onset of leukopenia has been noted, and the blood changes occur any time between the seventh and the sixtieth day of therapy and after ingestion of from 3.5 to 39.2 g. Neutropenia has developed four to six days after discontinuing the use of *phenylbutazone*, presumably because the drug is slowly metabolized and excreted and remains in the blood for as long as 10 to 21 days after

the last dose.<sup>482</sup> The incidence of neutropenia in association with use of this drug is uncertain. In some series an incidence as high as 0.6% was described<sup>479</sup> and many toxic side effects were recognized,<sup>482</sup> while others reported, especially when doses of less than 200 mg/day<sup>487</sup> were given, that little toxicity was experienced.<sup>478,487</sup> In New Zealand, an approximate incidence of 0.005% can be calculated from the amount of drug sold and the incidence of associated neutropenia.<sup>471</sup>

*Oxyphenbutazone* is said to have 40% more therapeutic effect<sup>478</sup> and less than half as much toxicity (all types) as phenylbutazone.<sup>478,487</sup> *Acetylsalicylic acid*, when used alone, has been associated with leukopenia in three instances.<sup>453</sup>

#### *Phenothiazines and Tranquillizers* (Table 41-7)

After the introduction of *chlorpromazine* in 1952, reports of "agranulocytosis" due to this drug began to appear. However, as mentioned earlier, it became apparent that the granulocytopenia associated with the use of chlorpromazine and related phenothiazines is of gradual onset, and readministration of a small dose of drug after recovery does not elicit an acute response like that seen following aminopyrine.<sup>509</sup> Neutropenia developed after the drug has been ingested in amounts of one to 52 g for periods of time varying from 13 to 171 days<sup>501</sup>; in 90% of subjects, neutropenia appeared during the first eight weeks of treatment.<sup>500</sup> Elderly Caucasian females seemed particularly susceptible,<sup>500,509</sup> and no case was seen in 1600 Negroes treated with chlorpromazine.<sup>503</sup>

The incidence of neutropenia was reported to be as high as 10.8% in a small group of elderly women treated with large doses of *mepazine*, but in other series of patients treated with this drug the incidence varied from 0.8 to 7%.<sup>500</sup>

The incidence of severe neutropenia in a large series of 6,200 psychiatric patients treated with a variety of the phenothiazine drugs was 1/1240 and transient mild leukopenia was seen in about one third of the

patients.<sup>506</sup> The neutropenia produced by phenothiazines appears to be due to suppression of marrow neutrophil production, as described above. Only rarely has a leukoagglutinin been found.<sup>503</sup> Several of the phenothiazines do not appear to produce neutropenia, eg, promethazine and methdilazine,<sup>506</sup> and in general it is believed that compounds with the highest potency per mg are least likely to produce neutropenia.<sup>507</sup>

*Meprobamate* also has been reported to cause leukopenia and agranulocytosis as well as other dyscrasias<sup>453,502</sup> on occasion.

#### *Sulfonamides (Antibacterial)*

Severe neutropenia has been reported in association with the use of a number of antibacterial sulfonamide drugs (Table 41-7). The incidence appears to be about 0.5 to 1%,<sup>523,527</sup> and neutropenia usually has developed after the drug had been given for a few days and in a substantial dose, eg, 40 to 50 g of sulfanilamide or sulfapyridine given over a period of 7 to 33 days.<sup>477,521,527</sup> However, severe neutropenia has occurred after much smaller doses (eg, 4 g), and sulfapyridine has been reported to have higher toxicity than sulfanilamide and sulfathiazole.<sup>527</sup> The association of skin rashes, fever, and allied manifestations, and the appearance of neutropenia after a few days of therapy,<sup>527</sup> or occasionally after intermittent therapy,<sup>530</sup> suggest a possible immunologic mechanism, but no clear case of a precipitous drop in blood neutrophils after exposure to a single small dose of drug has been reported.<sup>522,527</sup> A leukocyte antibody has been detected in one or two patients,<sup>522,528</sup> and it is suggested that an antibody activated by drug may act chiefly on marrow precursor cells to interfere with cell production.<sup>522</sup>

#### *Sulfonamides (Non-Antibacterial)*

Cases of neutropenia associated with antidiabetic and diuretic sulfonamide drugs<sup>521,529,526,528</sup> (Table 41-7) are rare, particularly considering the widespread use of these agents. The neutropenia appears to

come on after one of these drugs has been used for a considerable time. A leukoagglutinin was reported in one patient.<sup>525</sup>

### Antithyroid Drugs

In association with the administration of thiouracil the leukocyte count decreased to less than  $3.0 \times 10^9$  cells/l with slight to marked neutropenia in 3.4% of 1091 patients taking this agent.<sup>557</sup> Fever and infection in association with severe neutropenia occurred in 1.7% of these patients, and the death rate in those developing agranulocytosis was 26%.<sup>557</sup> Very similar findings have been reported by others.<sup>554, 558, 562</sup> The leukocyte count usually decreased only after four to eight weeks of treatment in previously untreated patients,<sup>557, 558</sup> but leukopenia was seen as early as two weeks after restarting the drug in patients who had taken it previously.<sup>557</sup> In a few of the patients the readministration of a single small dose of the drug was followed by a rapid fall in neutrophil concentration and/or the reappearance of toxic symptoms such as malaise, fever, and chills.<sup>559, 565</sup> In some patients taking thiouracil, moderate leukopenia developed but the leukocyte count then returned to normal with continued therapy.<sup>559, 565</sup>

Leukopenia has been reported to occur in 4% of patients treated with methylthiouracil and about 1% of patients developed agranulocytosis.<sup>559</sup> Others have reported a higher incidence of reactions.<sup>549</sup> Propylthiouracil appears to be somewhat less toxic with leukopenia, occurring in only about 1.5% and agranulocytosis in 0.5% of patients treated.<sup>551</sup> Antibodies against leukocytes were reported in patients sensitive to this drug.<sup>556, 561</sup> Methimazole therapy was associated with leukopenia in 2% and agranulocytosis in 1% of persons treated with this drug.<sup>553</sup> Carbimazole is thought to be one of the least toxic of the antithyroid drugs,<sup>552, 560</sup> particularly in regard to its effects on leukocytes,<sup>552</sup> but it has not been approved for use in the United States.

### Anticonvulsants

Anticonvulsants have been reported to cause leukopenia and neutropenia as well as thrombocytopenia or aplastic anemia. The incidence of slowly developing neutropenia in patients taking trimethyloxazolidine (Tridione) was as high as 6.3%, while 2% of patients receiving diphenylhydantoin developed mild leukopenia.<sup>574</sup> Other case reports of neutropenia associated with the use of several of the anticonvulsants can be found<sup>572, 573</sup> but the occurrence is unusual.<sup>571</sup> In practically all of the subjects, neutropenia developed only after at least several weeks of use of one of these drugs, was often heralded by a rash or fever, and the bone marrow showed decreased numbers of myeloid precursors.<sup>570</sup> No case in which the drug was readministered after the patient had recovered from severe neutropenia has come to our attention. The mechanism of the leukopenia may be similar to that seen with the phenothiazines, since phenylhydantoin decreased <sup>3</sup>H-uridine uptake by bone marrow cells obtained from patients recovered from phenylhydantoin leukopenia.<sup>567</sup>

### Antihistamines

These agents rarely cause leukopenia or agranulocytosis. In the few instances reported (Table 41-7), leukopenia and neutropenia appeared only after a period of at least five weeks of drug ingestion.<sup>580</sup>

### Antimicrobial Agents

A wide variety of antimicrobial agents have been reported to cause leukopenia or neutropenia, and, on more rare occasions, agranulocytosis. *Chloramphenicol* appears to be the antibiotic that most commonly affects leukocytes,<sup>457, 461</sup> but it is reported to cause aplastic anemia (Chapter 56) six or seven times as often as neutropenia.<sup>453</sup> Historically the *organic arsenicals* such as arsphenamine<sup>600</sup> and mapharsen<sup>595</sup> used in the treatment of syphilis were among the earliest antimicrobial

agents to cause neutropenia. They, too, caused aplastic anemia more frequently than single cytopenias.<sup>600</sup> The development of neutropenia often was gradual, in most instances appearing after several weeks or more of injections and often heralded by nausea, vomiting, fever, headaches, and other symptoms.<sup>592,595</sup> The incidence ranged from as low as only one or two cases in over 4,000 patients treated with mapharsen<sup>595</sup> to 12 cases of neutropenia in 1,882 patients thus treated. The occurrence of serious reactions leading to death appears to have been rare.

A number of agents, for example, *penicillin*,<sup>605</sup> *methicillin*,<sup>598,601</sup> *ampicillin*,<sup>594</sup> *para-aminosalicylic acid*,<sup>603</sup> *isonicotinic acid hydrazide*,<sup>589</sup> *ristocetin*,<sup>593,602</sup> *nitrofurantoin*,<sup>599</sup> *novobiocin*,<sup>604</sup> and *cephalothin*,<sup>597</sup> have been reported to cause neutropenia in one or two isolated instances. Cephalothin appeared to adhere to neutrophils (but not lymphocytes) from either normal subjects or the patient; the coated cells then presumably reacted with antibody, causing noncomplement-mediated destruction of the "innocent bystander" leukocytes.<sup>597</sup> *Tibione*, an antituberculous drug, caused neutropenia in about 1% of those treated with this drug.<sup>591</sup> *Metronidazole* caused neutropenia in about 2% of patients so treated, but the neutrophil count usually returned to normal even if used of the drug was continued<sup>606</sup> and no profound or dangerous reactions have been reported.<sup>596,606</sup> A few drugs, such as *lincomycin*, to date appear to have been incriminated on the basis of insufficient evidence.<sup>590</sup>

### Miscellaneous Drugs (Table 41-7)

A wide variety of other drugs and chemicals have been reported to occasionally cause neutropenia or agranulocytosis, although, with *phenindione*, the incidence of neutropenia was around 1%.<sup>639</sup> The neutropenia has usually developed after prolonged treatment,<sup>620,623,630,636</sup> and one drug, *procainamide*, that had produced neutropenia was even given again for some time before the neutropenia recurred.<sup>624</sup> An acute, immuno-

logic type of reaction was demonstrated in association with treatment with *mercurial diuretics*.<sup>625</sup>

### Idiopathic Reactions

It has not been possible to incriminate drugs in all cases of neutropenia or agranulocytosis. The proportion in which no etiologic agent was identified was as high as 38%<sup>461</sup> in some of the early experiences, but became much smaller as suspicion of a drug as the etiologic basis increased.<sup>457,460,483</sup> A careful history and repeated questioning of patients and family will often reveal a probable cause.<sup>638</sup>

### Symptomatology

In fulminating agranulocytosis, the onset is sudden and is marked by a chill, high fever, and necrotizing angina. Prostration is extreme. The patient often looks pale, yet the mucous membranes are of normal color or cyanotic. Jaundice is sometimes present. Gangrenous ulceration may be found on the gums, tonsils, soft palate, lips, tongue, pharynx, or buccal mucous membranes, and somewhat less frequently in the skin, nose, vagina, uterus, rectum, or anus. A dirty-yellow, gray or greenish-black membrane covers an underlying ulcer, but the surrounding tissue may show little reaction. Regional adenopathy is present in such subjects, but generalized lymphadenopathy does not occur. Splenomegaly and bone tenderness are very unusual and the liver is rarely enlarged. The fever is high, the pulse rapid and weak, and death ensues in a few days. Plum<sup>460</sup> noted that, with few exceptions, the duration of the illness in fatal cases was three to nine days. Three fourths of his patients died within three days following admission to the hospital.

When it is possible to obtain an adequate history, it is often found that a sensation of fatigue and overpowering weakness has preceded the onset of illness by two or three days. Sore throat may not appear until 12 to

24 hours after the onset of other symptoms.<sup>460</sup> Painful deglutition, headache, rigors, and chilly sensations are common and vomiting, dyspnea, mental confusion, and pain in different parts of the body may occur. Dermatitis or a rash has been observed in about 10% of the subjects.<sup>460</sup>

The train of events in patients with agranulocytosis due to drugs may be divided into three periods: (1) malaise, fever and perhaps a chill, prodromal symptoms that are often forgotten; (2) a period of freedom from symptoms except for fatigue and prostration, during which time the leukocyte count falls and the granulocytes disappear; (3) the final and frequently fatal stage when, resistance being lowered by the absence of granulocytes, infection occurs in those regions normally harboring bacteria and progresses with the extreme rapidity to be expected in defenseless tissues.

In other patients, as already discussed, the course is less rapid and the symptoms not so fulminating. These patients should perhaps not be classed with those presenting the typical Schultz syndrome

### The Blood

In typical agranulocytosis, deficiency in granulocytes is the outstanding finding, but usually other types of leukocytes also are reduced in number. In patients with fulminating cases the leukocyte count is usually less than  $2.0 \times 10^9$  cells/l and frequently it is below  $1.0 \times 10^9$  cells/l. Counts as low as  $0.05 \times 10^9$ /l have been recorded. The granulocytes may be completely absent or 1 to 2% may be found. These cells may possess a pyknotic nucleus and vacuolated cytoplasm with poorly staining granules. Myelocytes are seen only when recovery begins.

The majority of the cells that are to be found are lymphocytes. In some cases, monocytes may be increased relatively and even in absolute numbers. It was suggested that this represents a special type of agranulocytosis (*leukopenic infectious monocytosis*).<sup>378-379</sup> In other instances a number of cells of the Turk "irritation" type have been described.<sup>460</sup>

In chronic neutropenia of slow onset, the leukocyte count is rarely below  $2.0 \times 10^9$  cells/l and the granulocytopenia is less pronounced.

In patients with typical cases there is no anemia or thrombocytopenia and bleeding and coagulation times are normal. In a number of patients a slight or moderate degree of anemia has been observed, but in most of these there was no evidence that this did not exist before the presenting illness. The appearance of the red cells is normal and the number of reticulocytes is normal. The sedimentation rate, however, is greatly accelerated. The icterus index may be moderately raised.

### Bone Marrow

In the classic subject, the bone marrow, examined by biopsy or on postmortem, shows normal erythropoietic tissues and normal numbers of megakaryocytes. The picture as a whole may be one of moderate hypoplasia or of hyperplasia.<sup>461</sup> The striking feature often has been a lack of granulocytes, including polymorphonuclear cells, metamyelocytes, and myelocytes. The observation that promyelocytes and myeloblasts were found in the bone marrow at the time of examination led to the erroneous designation "maturation arrest."<sup>650</sup> Plasma cells, lymphocytes, and reticulum cells may be increased in number.<sup>618</sup>

### Other Laboratory Findings

The urine may contain traces of albumin but is otherwise normal. Blood cultures often have been positive and a great variety of organisms have been found in these as well as in throat cultures.

### Diagnosis

It is most important that the diagnosis be made in the early stage before septicemia has developed. However, the early symptoms are so much like those of many other illnesses that early recognition is unlikely unless the

physician has a high index of suspicion and obtains a leukocyte count whenever he is confronted with symptoms of unexplained weakness and profound exhaustion, or those of a severe acute infection. It is to be remembered that local symptoms in the buccal cavity may not always be present in agranulocytosis.<sup>460</sup> When drugs known to be associated with granulocytopenia are being employed, it is important that the physician be alert for the possible development of this syndrome.

Once the blood has been examined, it should not be difficult to rule out the possibility of the various types of pharyngitis and other infections occurring in the mouth or throat because these generally are accompanied by leukocytosis. Infections such as typhoid fever, measles, rubella, and undulant fever, which are associated with leukopenia, can be readily distinguished from agranulocytosis by their more gradual onset and characteristic symptoms and signs; but influenza is not so easily differentiated. In any of the conditions named it is most unusual for the leukocyte count to reach the low levels noted in agranulocytosis.

Other disorders that may be confused with agranulocytosis include acute "aleukemic" leukemia and aplastic anemia. These are usually accompanied by anemia and thrombocytopenia. The former usually is characterized by the presence of a few immature leukocytes in the blood as well as by adenopathy and splenomegaly. If doubt remains, bone marrow examination should make the diagnosis clear. Like agranulocytosis, infectious mononucleosis is marked by changes that are confined to the leukocytes but extreme leukopenia is unusual; the typical lymphocytes are found and reaction to the heterophil antibody test is positive. Several rare cases of agranulocytosis associated with infectious mononucleosis have been reported.<sup>653</sup>

### Prognosis and Course

Before the sulfonamides and antibiotics became available, the prognosis was very

poor. In Plum's series<sup>460</sup> of 88 patients, the mortality was 84%. In 7%, death occurred after temporary improvement. Statistics by other authors<sup>461</sup> gave the mortality as 70 to 90%. Unfavorable signs are confusion and drowsiness, great prostration, jaundice, necrosis of the skin, a leukocyte count less than  $1.0 \times 10^9$  cells/l with absence of all granulocytes, or the onset of complications such as pneumonia. Mortality is greatest in patients of advanced age.

Death may occur as the result of sepsis, pneumonia, hemorrhage following necrosis, or other causes even after hematologic improvement has set in. Before the importance of drugs as etiologic agents was recognized, relapses were common.

Improvement is heralded by the reappearance of leukocytes of the granular series in the blood. These at first are myelocytes and metamyelocytes. Myeloblasts may be seen. Segmented neutrophils are the last of this series to make their appearance. The presence of monocytes,<sup>461</sup> especially if they persist,<sup>654</sup> has been said to be a good sign. The leukocytic reaction may be very rapid and marked degrees of leukocytosis, even of "leukemoid" character, may be reached, although counts of about  $15.0 \times 10^9$  cells/l have been recorded most often.<sup>461</sup>

With the availability of potent antibiotics to control the complicating infection the outlook, even in severe cases, now is far better than when agranulocytosis was first discovered, provided the potential offending agent is sought out and its further use interdicted.

### Treatment

The most important measure is to discover, if possible, the *offending agent* and to *prohibit its further use*. In this regard it is important to warn patients given drugs that may cause granulocytopenia to discontinue their use immediately if any adverse reaction occurs and to report to the physician without delay. It is of almost equal importance to prevent overwhelming sepsis from developing before the leukocytes reappear. Suitable smears and cultures must be taken immedi-

ately in an effort to identify the organisms causing secondary infection and then a best guess must be made and treatment with the bactericidal drug or drugs most likely to be effective should be initiated. Once the results of culture and sensitivity studies are known, the therapy can be readjusted.

When the granulocytopenia is due to the action of arsenic or gold,<sup>626</sup> BAL (British Anti-Lewisite, 2,3-dimercaptopropanol) may be given intramuscularly, in doses of approximately 150 mg (1.5 ml) of a 10% solution in oil, every four hours for the first two days and then in this dosage twice daily for another eight to ten days. This agent will facilitate excretion of the toxic metal. Oral penicillamine (1 g/day in divided dose) also may be used and has been shown to enhance gold excretion.<sup>649</sup> It may avoid the complication of abscess or infection at injection sites that has occurred with BAL therapy.<sup>626</sup>

The general care of the patient is of great importance. Oral hygiene must receive particular attention in order to prevent the development of painful and serious ulcerations.

No means has been found to effectively stimulate granulocyte formation and, furthermore, it may be questioned whether the hematopoietic system is capable of producing these cells more rapidly than occurs once there is no further exposure to the causative agent.<sup>461</sup> The evidence that pentnucleotide<sup>652</sup> was valuable in the treatment of agranulocytosis was never convincing<sup>460,461,523</sup> and there certainly is no indication for its use now that potent antibiotics are available. Transfusions of blood from patients with leukemia<sup>646</sup> or with leukocytosis resulting from some infection were not found to be of value.<sup>460,652</sup> Injections of leukocytes, "leukocytic cream,"<sup>655</sup> liver extracts,<sup>650</sup> and various vitamins, the feeding of bone marrow, especially yellow marrow<sup>647</sup> and marrow extracts, and the irradiation of the long bones with "stimulating doses" of x rays<sup>651</sup> all had their brief popularity but never earned a place in the management of agranulocytosis. In recent years, considerable effort has been expended to develop methods for collecting fresh neutrophils in large enough numbers to make neutrophil transfusions therapeutically use-

ful. There are several problems.<sup>645</sup> (Chapter 12).

1. The short survival and rapid turnover of blood neutrophils render the effect of such transfusions evanescent at best.

2. Because neutrophils are fragile and easily damaged, collection techniques must involve minimal handling and only brief sojourn outside the body.

3. Large numbers of neutrophils must be given (eg, about  $5 \times 10^{10}$  cells just to replace the normal blood neutrophil pool) and thus the only practical donors are patients with chronic myelocytic leukemia. Such cells can and have been given safely and without transmission of leukemia, but whether they are of real benefit in agranulocytosis remains to be demonstrated.

4. The repeated transfusion of neutrophils may result in sensitization of the recipient. Some work has been done on typing leukocytes for transfusion, but such techniques are usually available only in large medical centers. The chances of finding compatible donors in the general population are remote, but if the patient has many siblings the chances are considerably improved.

In spite of the above difficulties, neutrophil transfusions are being used in some large medical centers to tide patients over periods of granulocytopenia induced by chemotherapy, and there is now some evidence that they are helpful (page 510).

In the classic case of acute agranulocytosis the use of adrenocorticosteroids or ACTH is not justified. The avoidance of continued exposure to a possible offending drug and the control of infections, as they arise, are far more important. In the absence of infection, no therapy is indicated. "Prophylactic" antibiotic administration to patients with chronic neutropenia is both unnecessary and dangerous; such patients may live without trouble for months and even years without infections developing.

## Pathology

In agranulocytosis the most significant feature revealed by autopsy is a lack of granulocytes. Polymorphonuclear leukocytes are



conspicuously absent about the necrotic lesions that may be found in the oral cavity, skin, vagina, uterus, or the gastrointestinal tract, and only plasma cells and lymphocytes are seen. Enormous collections of bacteria are to be found. In the lungs there may be pneumonic lesions with a similar lack of granulocytes, or gangrene. The liver is often enlarged and cloudy swelling, fatty degeneration, or small areas of necrosis may be present. The spleen is frequently somewhat heavier than normal. There is marked engorgement with blood, the lymph follicles are small, granulocytes are absent, and there may be small areas of necrosis. Of the lymph nodes, only those draining infected areas are enlarged. Microscopically they show hyperemia, hemorrhage, and necrosis with some obliteration of the normal structure.

The bone marrow was described on page 1298.

## Leukemoid Blood Pictures

Patients with blood findings that resembled some type of leukemia, but in whom leukemia was not confirmed by the subsequent course of the illness or at autopsy, have been referred to as exhibiting a leukemoid blood picture. More restrictive definitions have been proposed, such as those which require a leukocyte count greater than  $50 \times 10^9$  cells/l or the presence of a certain proportion of blasts in the blood,<sup>681,682</sup> but these have not facilitated classification or understanding since their use excludes some cases that, in fact, simulate leukemia. In some patients, only the magnitude of the leukocytosis or the presence of young forms suggested leukemia<sup>672,689</sup>; in others, the presence of anemia or thrombocytopenia made the differentiation more difficult. In spite of the confusing blood findings the clinical diagnosis has not been difficult in most cases.<sup>672,689,692</sup> In a few the presence of adenopathy, splenomegaly, fever, or hemorrhage made exclusion of leukemia difficult or impossible.<sup>667,709</sup> Not infrequently the true diagnosis was made only at autopsy in such patients,<sup>667</sup> and differentiation was not always possible even then.<sup>686,692,709,715</sup>

**Table 41-8. Diseases Most Commonly Giving Rise to a Leukemoid Blood Picture**

- 1 Infections presenting pictures resembling  
Myelocytic or myeloblastic leukemia pneumonia, diphtheria, tuberculosis  
Lymphocytic leukemia<sup>7</sup> whooping cough, chickenpox, infectious mononucleosis, infectious lymphocytosis, tuberculosis  
Monocytic leukemia tuberculosis
- 2 Intoxications eclampsia, severe burns, mercury poisoning
- 3 Malignant disease, especially with bone metastases also multiple myeloma, myelofibrosis, Hodgkin's disease
- 4 Severe hemorrhage, sudden hemolysis of blood

Leukemoid reactions occur in association with a variety of infections, intoxications, malignant diseases, and even severe hemorrhage or sudden hemolysis (Table 41-8). Bone marrow aspiration and biopsy, and liver and lymph node biopsy, together with careful examination of these materials by culture and special stains, should be helpful in differential diagnosis.<sup>684</sup> It is apparent that unusually high leukocyte counts or immature cells in the blood must be the consequence of abnormalities in production or release of cells, possibly coupled with defective removal and destruction. However, little is known about the mechanisms that control these events even in the normal state (page 253).

*Myeloid leukemoid blood pictures* may be considered under several headings:

1. Exceptionally high leukocyte counts (mainly neutrophils) with little immaturity and few if any other features to suggest leukemia. Such findings have been encountered in Hodgkin's disease ( $54.4 \times 10^9$  cells/l),<sup>681</sup> non-metastatic gastric carcinoma with infection ( $110.0$ ),<sup>700</sup> acute infection with hemorrhage in advanced breast carcinoma ( $120.0$ ),<sup>692</sup> carcinoma of the lung ( $93.0$ ),<sup>672,714</sup> adrenal carcinoma with metastases ( $88.0$ ),<sup>683</sup> retroperitoneal fibrosarcoma ( $82.0$ , personal observation), alcoholic fatty liver ( $38.9$ ),<sup>666</sup> acute glomerulonephritis ( $51.9$ ),<sup>682</sup> dermatitis herpetiformis ( $68.0$ ),<sup>671</sup> and acute rheumatoid arthritis ( $86.0$ ).<sup>664</sup> Such

patients seldom if ever present any serious difficulty in clinical diagnosis.

2. Leukocytosis of variable degree with a considerable left shift in the differential count including myelocytes, promyelocytes, and a few blasts, thus producing a blood picture similar to that of chronic myelocytic leukemia (CML). This has been reported in association with a variety of tumors, many with metastases to bone.<sup>693,694</sup> In most of these patients the leukocyte count was normal or only moderately increased ( $<17.0 \times 10^9$  cells/l), and myeloid immaturity increased with increasing anemia and bone involvement.<sup>699</sup> Disseminated tuberculosis also has produced a picture that resembled CML with counts as high as  $220.0 \times 10^9$ /l,<sup>697,694,707</sup> as have a variety of other infections including staphylococcal pneumonia (114.0), meningococcal and hemophilus meningitis, salmonella and paracolon sepsis, pneumococcal endocarditis (76.8), diphtheria (70.0), bubonic plague (100.0),<sup>692</sup> and infected abortion (112.0),<sup>692</sup> as well as lymphoma,<sup>691</sup> eclampsia,<sup>696</sup> rheumatoid arthritis,<sup>693</sup> and heavy metal (Hg) or drug toxicity.<sup>670,681</sup> The use of ancillary diagnostic aids such as the leukocyte alkaline phosphatase stain, serum vitamin B<sub>12</sub> level, and karyotype analysis for the Ph<sub>1</sub> chromosome would be expected to be helpful in those few cases in which real difficulty in differentiation from CML exists,<sup>694</sup> but confusing results have sometimes been obtained.<sup>684,714</sup> Myelofibrosis and osteosclerosis frequently produce a blood picture that may simulate CML, but the red cell changes often suggest the diagnosis and marrow biopsy provides confirmation (page 1779).

3. A leukemoid picture simulating acute myeloblastic leukemia has been associated with disseminated tuberculosis or tuberculosis involving the spleen and lymph glands,<sup>693,697,707,708,709,712</sup> and has been observed when the bone marrow was regenerating in patients with agranulocytosis and superimposed infection, whether bacterial<sup>692,695</sup> or protozoan.<sup>687,702</sup> A picture simulating aleukemic, myeloblastic leukemia has been described in alcoholics with infec-

tion. There was neutropenia and a mild shift to the left in the blood, and the bone marrow revealed few neutrophilic precursors more mature than promyelocytes.<sup>699</sup> The leukopenia lasted only several days after hospitalization and a resurgence of granulocytopoiesis with band and segmented forms was evident in the marrow by four days. In these alcoholics the blood leukocyte count failed to rise following injection of endotoxin,<sup>699</sup> thus indicating decreased marrow granulocyte reserves.<sup>699</sup> Several similar cases have been described in association with megaloblastic anemia of the puerperium<sup>704</sup> and we have seen such a reaction in a patient with pernicious anemia. Such AML-like reactions suggest that, with poor marrow granulocyte reserves for whatever reason (drug suppression, folate or B<sub>12</sub> deficiency, alcoholism, etc.), a severe infection exhausts the marrow stores and leukopenia with a variable degree of shift to the left develops. As restoration of myelopoiesis begins the appearance of the marrow is not unlike that of myeloblastic or promyelocytic leukemia.<sup>687,695</sup> The best approach in such cases is to treat any infection aggressively with bactericidal antibiotics after obtaining appropriate cultures, to supply vitamin B<sub>12</sub> and folate parenterally if the patient may be nutritionally deficient, and to avoid anti-leukemic therapy until it is clear that one is not dealing with a leukemoid reaction.

The presence of Auer rods has been reported in patients with AML-like leukemoid reactions associated with disseminated tuberculosis.<sup>709</sup> In these patients, even though no evidence of leukemia was found at autopsy, tuberculosis and acute leukemia may have coexisted since, it is claimed, tuberculosis may so modify the morphologic features of leukemia that the characteristic leukemic infiltrations are not demonstrable.<sup>684</sup>

Lymphoid leukemoid reactions also may be of several types:

1. A high lymphocyte count consisting mostly of mature lymphocytes, thus simulating chronic lymphocytic leukemia (CLL), has been most commonly found in infants

and young children with *pertussis*. Leukocyte counts as high as  $272 \times 10^9$  cells/l have been reported. The prognosis in such cases was regarded as serious,<sup>660,710</sup> particularly in the pre-antibiotic era.<sup>680,711</sup> Differentiation from CLL is easily made on the basis of the youth of the patients and the lack of adenopathy and splenomegaly. *Infectious lymphocytosis* also results in a high lymphocyte count (page 1289). The young age of the patient and the presence of fever, as well as the frequent involvement of several family members, are helpful differential features. A CLL-like blood picture has been associated with other diseases as well, eg, dermatitis herpetiformis,<sup>671</sup> exfoliative dermatitis,<sup>675</sup> chickenpox,<sup>679</sup> cancer of the stomach,<sup>663</sup> metastatic melanoma,<sup>691</sup> breast cancer,<sup>690,703</sup> and miliary tuberculosis.<sup>677</sup> The simultaneous presence of CLL or non-Hodgkin's lymphoma and one of these diseases cannot be ruled out in some of these cases since the patients were not always observed for long periods and some received x-ray therapy.

2. When numerous young mononuclear cells have been present in the blood, whether the leukocyte count was low, normal, or increased, *acute lymphoblastic leukemia* (ALL) has been simulated. The most common disorder leading to confusion with ALL is infectious mononucleosis (Chapter 43). Although leukocytosis greater than  $30.0 \times 10^9$  cells/l occurs in less than 1.5% of patients,<sup>662</sup> counts up to  $80.0 \times 10^9$  cells/l have been reported.<sup>674,703</sup> and we have seen one patient with  $100.0 \times 10^9$  cells/l. A similarly confusing picture has been reported in infectious hepatitis, in the post-transfusion syndrome, and in some cases of drug sensitivity,<sup>713</sup> as well as in association with mumps,<sup>676</sup> congenital syphilis,<sup>668</sup> tuberculosis,<sup>696,709</sup> and a variety of other illnesses. It should be noted that young-appearing, DNA-synthesizing cells may enter the blood in response to both inflammation and antigenic stimulation. Some of these cells may be lymphocytes while others may belong to the monocytic series.<sup>371</sup>

*Other varieties of leukemoid reaction have*

been reported, but they are very rare. Only one case of *monocytic leukemoid* reaction has been reported and this was associated with tuberculosis of the lymph glands, lungs, liver, and ileum and a mediastinal teratoma. The leukocyte count reached  $82 \times 10^9$  cells/l with 42% monocytes. This patient died five months after becoming ill and it is difficult to rule out the possibility of the concurrence of leukemia.<sup>678</sup>

*Eosinophilic leukemoid* reactions with high concentration of eosinophils in the blood have been reported in association with amebiasis<sup>688</sup> and melanomatosis<sup>701</sup> as well as in cases which simulated (and may have been) chronic eosinophilic leukemia<sup>698</sup> (page 1285).

### Experimental Production of Leukemoid Reactions

The administration of tuberculin to previously sensitized rabbits has elicited a leukemoid reaction with leukocyte concentrations reaching  $124 \times 10^9$  cells/l. The reaction was predominantly granulocytic with considerable shift to the left<sup>673</sup>; monocytic response was minimal. Leukemoid reactions with white cell counts (chiefly neutrophils) of several hundred thousand have been reported in mice harboring several types of transplantable tumor.<sup>661,669</sup> Resection of the tumor reduced the leukocyte count and if the tumors recurred so did the leukocytosis. A substance that appears to stimulate granulocyte production and release was isolated from one such tumor<sup>669</sup> (page 253). It has been suggested that the presence (and presumably overgrowth) of leukovirus in animals with weakened body defenses may result in leukemoid reactions.<sup>701</sup>

The *lymphocytic leukemoid* reaction produced by pertussis has been studied in mice. It appears that pertussis organisms liberate a substance that attaches to lymphocytes and prevents their "homing" to lymphoid organs; lymphocytosis develops due to decreased egress of lymphocytes from the blood.<sup>706</sup>

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## *Quantitative, Morphologic, and Functional Disorders of the Granulocyte and Monocyte-Macrophage Systems*

**Quantitative Disorders of Granulocytes**  
**Disorders of Phagocytic Leukocytes Characterized by Morphologic Changes**  
**Functional Disorders of Leukocytes Not Characterized by Morphologic Changes**  
**Disorders Involving the Monocyte-Macrophage System—The "Storage Diseases"**  
**Histiocytosis X**

**I**N Chapter 41, variations in the numbers and morphologic characteristics of leukocytes occurring in association with disease or exposure to drugs were described and their significance was discussed. To be considered in the present chapter are alterations in the granulocyte and monocyte-macrophage systems that mainly represent (1) abnormalities in production, destruction, or control mechanisms that result in leukopenia (neutropenia) or neutrophilia; (2) abnormalities of the cells of these systems that produce detectable morphologic changes and may or may not affect the cells' function; (3) enzymatic deficiencies that result in the accumulation of incompletely digested metabolic products (the "storage diseases"); or (4) other disorders characterized by functional abnormalities not associated with prominent morphologic changes, such as those leading to chronic granulomatous disease of childhood.

Many of these conditions are rare, even extremely rare. A number are inherited, not infrequently arising out of consanguineous marriages. A few are acquired. Study of some of these conditions, such as chronic granulomatous disease, Chediak-Higashi syndrome, myeloperoxidase deficiency, and the storage diseases, has provided considerable information about the normal function of leukocytes and macrophages.

### **Quantitative Disorders of Granulocytes**

From time to time, cases of neutropenia do not fit well into the recognized categories discussed in Chapter 41. Certain special features seem to distinguish them from one another, and one feature is common to them all—they are very poorly understood. They illustrate how fragmentary is our knowledge of the factors governing the numbers of leukocytes that are produced, circulate in the blood, and are destroyed.

Classification of these neutropenias on the basis of mode of inheritance or kinetic mechanisms has been largely thwarted by lack of adequate information. The result is a "pot-pourri" of clinical syndromes.

### Infantile Genetic Agranulocytosis<sup>9</sup>

Infantile genetic agranulocytosis is inherited as an autosomal recessive trait and is characterized by severe neutropenia ("almost complete agranulocytosis"); it is accompanied by frequent infections of various kinds, most commonly furuncles and carbuncles. The outcome usually is death within the first year of life. In the first study reported, consanguinity of the parents was established in five of nine families.<sup>9</sup> Symptoms may begin as early as one to three weeks after birth. Although infections may clear up with intensive antibiotic treatment, they soon recur. The neutropenia is severe, fewer than  $0.5 \times 10^9$  neutrophils/l usually being found. Total leukocyte counts often give values within the normal range and monocytosis is prominent in some subjects. Platelet concentration is normal, but moderate anemia is the rule. Examination of the bone marrow reveals variable cellularity (normal,<sup>15</sup> increased,<sup>5</sup> or decreased<sup>9</sup>); or few or no neutrophils, more mature than myelocytes, are present and yet mature eosinophils and basophils are found.<sup>15</sup> Vacuoles and atypical nuclear shapes are prominent in the promyelocytes and myelocytes, and atypical erythroid forms also have been noted.<sup>9</sup> Electron microscopy did not establish the nature of the vacuoles.<sup>5</sup> Culture of marrow cells from one patient resulted in increased maturation when normal serum or cysteine was added to the medium. Thus it was suggested that the basic defect may be inability to utilize sulfur-containing amino acids,<sup>9</sup> but administration of this amino acid produced no clinical improvement. Other studies of marrow particles cultured *in vitro* showed no or very slow maturation of neutrophils.<sup>15</sup> Patients' marrow cells dispersed in agar plates exhibited normal growth in some studies<sup>3</sup> and abnormal growth in others.<sup>11a</sup> Chromosome analyses have revealed no abnormality in most cases studied.<sup>7</sup> Normal neutrophils transfused into affected patients left the blood at approximately normal rates, thus suggesting that the defect is in cell production rather than due to increased cell destruction. The infusion of

normal plasma did not alleviate the neutropenia or alter the appearance of the bone marrow.<sup>15</sup>

Sporadic cases thought to represent the same disorder have been reported.<sup>1,2,7,10,11,12,13,15</sup> Hyperglobulinemia was noted in many of the subjects.<sup>7,11,12,15</sup> Some of the reported patients have survived into the late teens,<sup>7,12,15</sup> apparently because monocyte function is preserved and compensates for the missing neutrophils.<sup>15</sup> Treatment with steroids,<sup>7,15</sup> testosterone,<sup>11</sup> and/or splenectomy<sup>7</sup> has not been helpful.<sup>7,15</sup> One patient developed acute leukemia at age 13.<sup>7</sup>

### Familial Neutropenia Caused by Deficiency of a Plasma Factor<sup>20,22</sup>

Two brothers, ages 13 and 10, born to healthy and unrelated parents who had two healthy daughters, were noted to suffer from periodontal disease and frequent infections of the throat. Neutropenia with a shift to the left was found and the bone marrow showed "maturation arrest" at the myelocyte level and prominent eosinophils. Erythropoiesis was also thought to be abnormal. This picture is similar to that of infantile genetic agranulocytosis except for the longer survival and occasional transient rises in total leukocyte and granulocyte concentrations in association with infections. The plasma from the two boys inhibited differentiation of normal marrow cells *in vitro*. In addition, transfusion of normal serum led to short-term normalization of myelopoiesis in one boy, while in the other the effect was equivocal.

### Granulocytopenia with Associated Immunoglobulin Abnormality<sup>29</sup>

#### Familial Form

Three young brothers died at ages 3, 27, and 41 months, respectively, from a disease characterized by ulcerative stomatitis and pharyngitis, repeated respiratory infections, neutropenia, lymphadenopathy, splenomegaly, and hypogammaglobulinemia. The fact that their two sisters remained healthy

suggests that X-linked recessive inheritance was the basis for their disease. Their bone marrow showed arrested maturation at the myelocyte level, the majority of the granulocytes being promyelocytes and myelocytes. However, a few metamyelocytes were present and occasionally complete, temporary recovery of the blood and marrow neutrophil levels took place. A search was made for antibodies of several varieties but none was found. Skin window studies revealed a delayed and hypocellular response and, according to the few details reported, the phagocytic ability of the patients' leukocytes appeared to be reduced. At necropsy, extensive focal organizing pneumonitis was present without evidence of granuloma formation. No organisms were identified. The inflammatory response was minimal although in one of the boys small abscesses composed of young neutrophils were seen. The lymph nodes and thymus were relatively normal.

### **Nonfamilial Form**

This category includes six of eight patients with agammaglobulinemia who exhibited neutropenia and an associated shift to the left in the marrow neutrophil picture.<sup>26</sup> The neutropenia was transient in three of them, chronic in two, and appeared to be cyclic in one. Another patient with decreased 7S gammaglobulins and increased 19S globulins exhibited intermittent neutropenia that seemed to disappear as long as he was given gamma globulin injections monthly.<sup>25</sup> A similar patient with absent  $\beta$ -2A globulin and increased  $\beta$ -2 macroglobulin was studied with  $^{32}\text{P}$ -labeled neutrophil infusions; the  $t_{1/2}$  was four hours and a combination of increased cell destruction and inadequate marrow compensation was suggested.<sup>27</sup> Several bone marrow examinations revealed almost no plasma cells.

### **Congenital Aleukia or Reticular Dysgenesis<sup>35</sup>**

This very rare disorder was reported in newborn twin brothers who had no blood

leukocytes of any kind; the erythroid and megakaryocytic systems were normal. No antibodies against leukocytes were found in either the mother or the boys. In both boys, at necropsy virologic studies of body fluids and tissues revealed no abnormality; lymph nodes, tonsils, and Peyer's patches were absent and there were no lymphocytes, plasma cells, or follicles in the spleen. The bone marrow showed normal erythroid development and megakaryocytes, but no myeloid cells of any stage could be identified. It appears that congenital aleukia reflects lack of development of the leukocyte systems at the most primitive level and of the most complete degree.

### **Familial Benign Chronic Neutropenia<sup>41</sup>**

Familial benign chronic neutropenia is a rare, relatively benign anomaly that is transmitted as a non-sex-linked dominant trait and is characterized by normal or somewhat low total leukocyte counts, consistent neutropenia, and, usually, relative monocytosis and lymphocytosis, sometimes with eosinophilia. The red cell and platelet systems apparently are normal. The bone marrow is normally cellular, but few myeloid cells more mature than myelocytes are seen.<sup>40,41,44</sup> Clinically the disorder usually is detected by chance. In some affected family members, periodontal disease, a tendency to develop frequent furuncles, and limited neutrophil response to infection have been noted, but in others there appear to be no deleterious effects or symptoms.<sup>41</sup> Dominant transmission, the milder clinical course, and lack of involvement of the erythroid system distinguish this disorder from infantile genetic agranulocytosis.

Neutropenia occurring in family members has been reported under the title "familial benign chronic neutropenia" and may have been the same process as that described in the preceding paragraph. However, the manifestations differed in various ways; eg, there was no shift to the left in the bone marrow and the entity was confined to Yemnicite Jews.<sup>43</sup>

### Transitory Congenital Neutropenia Due to Transplacental Transmission of Neutropenic Factor (Isoimmune Neonatal Neutropenia)

This neutropenia was reported in two infants born of neutropenic mothers.<sup>63</sup> Both infants recovered spontaneously in several weeks. In the mother of one of these children a neutropenic factor could be detected in the serum by leukoagglutination tests or by the production of neutropenia in a normal recipient given the mother's plasma. The factor was associated with the gamma globulin fraction and caused "maturation arrest" in the bone marrow of the normal recipient.<sup>63</sup> In the other child whose mother was neutropenic, the only evidence for a neutropenic factor was the presence of a transitory neutropenia. The reason for the development of these "immune" factors is not clear since no associated disease could be identified. However, fetal leukocytes have been found in maternal blood, especially during the first trimester and after delivery.<sup>64</sup> Furthermore, the congenital neutropenia appeared in the first child of a mother who previously had had no blood transfusions.<sup>63</sup> One case was reported in an infant born to a pancytopenic mother who had lupus.<sup>62</sup>

Under titles similar to the above a number of infants with neutropenia have been described,<sup>53, 56, 59</sup> but the mothers of these infants were not neutropenic and the infants were the products of second or later pregnancies. Frequently, several siblings were affected. It was proposed that maternal isoimmunization might explain this form of neutropenia just as has been demonstrated in erythroblastosis<sup>55, 56, 59</sup>; subsequent studies have provided good evidence for this, at least in some instances.<sup>51, 57</sup> Several antigens (NA1 and NB1) peculiar only to the neutrophil have now been reported and apparently explain the occurrence in several families of neonatal neutropenia that resulted from fetomaternal incompatibility.<sup>57</sup> However, the incidence of neonatal neutropenia secondary to transplacental passage of leukocyte agglutinins must be low since in two series of infants (39<sup>50</sup> and 44,<sup>60</sup> respectively) in whose

mothers leukoagglutinins were found, no instances of neonatal neutropenia were detected. This entity is now referred to as *isoimmune neonatal neutropenia*.<sup>57</sup>

### Chronic Granulocytopenia of Childhood<sup>65</sup>

Chronic granulocytopenia of childhood is a relatively benign, apparently nonfamilial disorder characterized by repeated pyogenic infections beginning soon after birth. In at least some of these patients, complete recovery eventually takes place. Leukocyte concentration is usually normal, but neutrophil counts have ranged from 0 to  $2.0 \times 10^9/l$ ; monocytosis is common. Bone marrow study has revealed normal or moderately increased cellularity with some increase in lymphocytes; neutrophilic bands and metamyelocytes have been plentiful, but mature segmented forms have been absent. In contrast to patients with infantile genetic agranulocytosis, the blood neutrophils of children with chronic granulocytopenia increase in response to infection or to pyrogen or epinephrine injection. The marrow mitotic index is increased and marrow specimens grown in culture exhibit maturation of band forms into segmented neutrophils. For these reasons this disorder has been thought to be due to increased destruction of neutrophils. However, no kinetic studies have been reported. Attempts to detect leukoagglutinins have yielded negative results. Skin window studies showed delayed appearance of cells during the first seven hours.

The main differences between these patients and children with infantile genetic agranulocytosis are: in the former, a milder clinical course, less severe neutropenia, the ability to mobilize some neutrophils, and the presence of myeloid cells through to band forms in the marrow.

### Periodic or Cyclic Neutropenia<sup>65</sup>

The cyclic recurrence of malaise, fever, mild infection (often stomatitis with oral ulcers), and cervical lymphadenopathy has been recorded since 1910. The association of



these symptoms with neutropenia is well established.<sup>86,87</sup> In the majority of patients with this rare disorder, symptoms have begun in infancy, thus suggesting a congenital origin; in a few, the symptoms have begun later, even in the sixth and seventh decades of life. Cases of cyclic neutropenia affecting several family members have been reported.<sup>71,79,83,90</sup> Episodes recur at approximately three-week intervals (12 to 35 days, average 21 days) and symptomatic periods may last from three or four to ten days. During the symptomatic period the blood neutrophil concentration is markedly reduced and lymphocytosis, monocytosis, and/or eosinophilia has been noted in some patients.<sup>85,87</sup> The total leukocyte count may fluctuate with the neutrophil changes or may remain more or less constant in the low normal range.<sup>85,90</sup> Thrombocytopenia has been reported in a few cases.<sup>86</sup> Between attacks, the symptoms disappear completely and the neutrophil count returns to nearly normal levels. However, in most of the subjects the proportion of segmented neutrophils remains less than 50% and sometimes it is less than 20%.

Studies of the marrow at several points in the cycle have demonstrated that granulocyte precursors disappear prior to the onset of neutropenia and reappear before neutrophils reappear in the blood.<sup>81,85</sup> This led most observers to regard the clinical picture as secondary to a periodic failure of neutrophil production.<sup>85,86</sup> This hypothesis has been further supported by (1) failure to find leukoagglutinins in the serum; (2) inability to inhibit *in vitro* neutrophil migration or phagocytic capacity by relapse phase serum; or (3) failure to produce neutropenia or clinical manifestations in persons transfused with patient's serum.<sup>85</sup> Tritiated thymidine and  $DF^{32}P$  kinetic studies have provided more firm evidence against increased neutrophil destruction in the periphery and in favor of a periodic failure or inhibition of neutrophil production in the marrow.<sup>77,81</sup> No relationship to hormone levels or to the menstrual cycle has been established.<sup>85</sup>

The existence in gray collie dogs of a similar syndrome, except that the average cycle runs 8 to 12 days, has made more extensive

pathogenetic study possible.<sup>75,80,88</sup> In these animals, cyclic neutropenia is inherited as an autosomal recessive disorder.<sup>80</sup> Detailed studies of the blood revealed a cyclic pattern of blood neutrophils, eosinophils, monocytes, reticulocytes, and platelets with a wave of myelopoiesis in the marrow preceding the reappearance of neutrophils in the blood.<sup>75,80</sup> These studies, together with demonstrated reduction in marrow reserves before the development of neutropenia and normal  $DF^{32}P$  neutrophil half-disappearance time during the neutropenic phase, all indicate that the condition results from a recurring failure of myelopoiesis at a primitive cell level.<sup>75</sup> Demonstration of cyclic fluctuation of a urinary factor that stimulates myeloid colony growth in culture plates *in vitro* (CSA, see page 254, Chapter 6) may provide further support for this hypothesis.<sup>74</sup> Transplantation of normal marrow cells to a gray collie dog resulted in normal granulocytopoiesis, thus demonstrating that this disorder reflects a genetic stem cell defect.<sup>76</sup>

The course of the disorder in affected individuals (either man or dog) is marked by recurrent infections with periods of well-being interspersed. Treatment with antibiotics may decrease the severity of bacterial infections and reduce the likelihood of dissemination and death. Splenectomy, although not eliminating the cycling, has been reported to result in fewer symptoms in older patients and in those with splenomegaly. In addition, treatment with adrenal corticosteroids and androgenic hormones has at times been reported to be of some benefit.<sup>70,72</sup>

### Chronic Idiopathic Neutropenia

Chronic idiopathic neutropenia is a clinically benign, chronic syndrome that has persisted in those afflicted for 1 to 19 years and is associated with little or no increase in infections, although gingivitis is prominent in some subjects. It is characterized by severe neutropenia ( $0$  to  $0.8 \times 10^9$  cells/l) and a cellular marrow with normal myeloid maturation but lacking segmented neutrophils. Splenomegaly has not been found. Adrenal corticosteroids have not produced an



Fig. 42-1. Marrow specimen from a patient with "myelokathexis." Note the pyknotic nuclei, cytoplasmic vacuoles, and hypersegmented neutrophils with long thin, intrasegmental chromatin strands (From Krill et al.<sup>115</sup> courtesy of the authors and the New England Journal of Medicine.)

increase in neutrophil count nor did splenectomy change the course in one patient so treated.<sup>95</sup> Leukoagglutinins were absent in 13 of 15 patients; the remaining two patients were multiparous women. No treatment appears to be needed for these patients.

### Chronic Idiopathic Immunoneutropenia in Adults

Descriptions have been published of a number of cases of chronic neutropenia in which factors capable of agglutinating normal leukocytes *in vitro* were present in the patients' blood plasma.<sup>100, 101, 102, 103, 105, 106, 107, 108</sup>

In a few instances the patient's plasma was given to a normal recipient whose leukocyte and granulocyte counts dropped as a result.<sup>101</sup> In some of the patients, elevated levels of immunoglobulins of various types that were thought to be of pathogenic significance have been described.<sup>102, 103, 105, 106</sup> The bone marrow picture usually was a cellular one with a variable paucity of the more mature neutrophil forms, thus suggesting "maturation arrest" at the myelocyte stage.<sup>101</sup>

### "Myelokathexis" or Chronic Idiopathic Granulocytopenia

One case of chronic idiopathic granulocytopenia, apparently resulting from intramedullary retention and death of neutrophils,

has been reported.<sup>115, 116</sup> The patient was a 10 year old girl who had suffered from repeated infections and persistent neutropenia since infancy. Numerous bone marrow examinations revealed no "maturation arrest" and segmented neutrophils were present in abundance. However, pyknotic nuclei, cytoplasmic vacuoles, and hypersegmentation with longer than normal chromatin strands separating nuclear lobes were noted (Fig. 42-1). According to DF<sup>32</sup>P kinetic studies, the  $t_{1/2}$  of this patient's neutrophils was shortened in her own circulation and in the circulation of a normal recipient, while normal cells survived about normally in the patient's circulation.<sup>115</sup> Although cells were released from the marrow into the blood after stimulation with endotoxin or in response to infection, the patient's polymorphonuclear neutrophils exhibited decreased motility, decreased phagocytic capacity, and increased permeability to dyes. All of these findings seem to support the hypothesis that the mature granulocytes were functionally and morphologically inferior, that many were retained and died in the marrow, and that the neutropenia and clinical picture were the consequence of this defect.

### The Lazy Leukocyte Syndrome

Two unrelated children (a girl 4½ years and a boy 2½ years of age) who suffered from

recurrent stomatitis, gingivitis, and other infections associated with severe neutropenia but with cellular marrows containing normal numbers of mature neutrophils have been described.<sup>120</sup> Relatively few cells were released into the blood following endotoxin injection, but neutrophil morphology, random mobility, and phagocytic capacity were normal. Opsonin activity and complement-mediated chemotaxis were generated normally by the patients' plasma, but their neutrophils showed deficient response to their own chemotactic factor as well as to that generated in control sera. In another case, neutrophilia and poor cell-spreading on glass with narrow pseudopods, as well as ultrastructural and biochemical abnormalities, suggest a defect in actin filaments.<sup>119</sup> Still other examples of disorders of chemotaxis are being described.<sup>121,123</sup>

### Chronic Hypoplastic Neutropenia<sup>127</sup>

This syndrome is characterized by chronic neutropenia and absolute granulocyte counts often below  $0.7 \times 10^9/l$ , but occasionally rising above 1.8, into the normal range. The total leukocyte count usually is normal and lymphocytosis and/or monocytosis is present. At times, slight anemia or thrombocytopenia has been noted. These patients suffer from repeated infections that are slow to heal, the skin and oral cavity being most frequently affected. Splenomegaly of slight to moderate degree was present in the four patients reported,<sup>127</sup> but the lack of response to splenectomy was thought to exclude the possibility of "primary splenic neutropenia" (see below). Other than neutropenia the outstanding feature was a *remarkable hypoplasia of the entire granulocytic series* in the marrow with relatively undisturbed erythropoiesis and thrombocytopoiesis. No signs of inflammatory, malignant, or other disease that might produce this picture could be found. In one patient the process continued for 25 years. Treatment with immunosuppressive agents has not been helpful.<sup>125</sup>

### Primary Splenic Neutropenia

Primary splenic neutropenia is characterized by neutropenia of variable but often

mild degree ( $1.0$  to  $2.0 \times 10^9$  cells/l), splenomegaly, and myeloid hyperplasia of the marrow; it was said to be cured by splenectomy.<sup>132</sup> The spleen is described as showing extensive reticular hyperplasia, and phagocytosis of blood cells by reticulum cells may be noted. Some degree of anemia or thrombocytopenia may be associated, and in some subjects pancytopenia may occur ("splenic pancytopenia").<sup>130</sup> The relationship between this syndrome and cytopenias associated with Felty's syndrome, Banti's syndrome, and tropical splenomegaly is not at all clear.<sup>130,132</sup>

### Hereditary Neutrophilia

A mother and three of her four children have had life-long, persistent neutrophilia ( $9$  to  $62$  neutrophils  $\times 10^9/l$ ), high leukocyte alkaline phosphatase scores and vitamin  $B_{12}$  levels, hepatosplenomegaly, and Gaucher-like cells in the spleen and marrow, but morphologically and functionally normal neutrophils.<sup>135</sup> An autosomal dominant disorder is postulated.

## Disorders of Phagocytic Leukocytes Characterized by Morphologic Changes

For the most part the disorders to be considered here are rare, usually familial, and often reflect a general metabolic defect whose major manifestation may be more serious in tissues other than in leukocytes. Nevertheless, the leukocytic morphologic abnormalities may come to the attention of the hematologist and familiarity with them and the associated diseases may facilitate diagnosis.

### Pelger-Huët Anomaly

This benign anomaly of leukocytes is inherited as a non-sex-linked, dominant trait. It is characterized by distinctive shapes of the nucleus of leukocytes, by a reduced number of nuclear segments, best seen in the neutrophils, and by coarseness of the nuclear chromatin of the neutrophils, lymphocytes, and

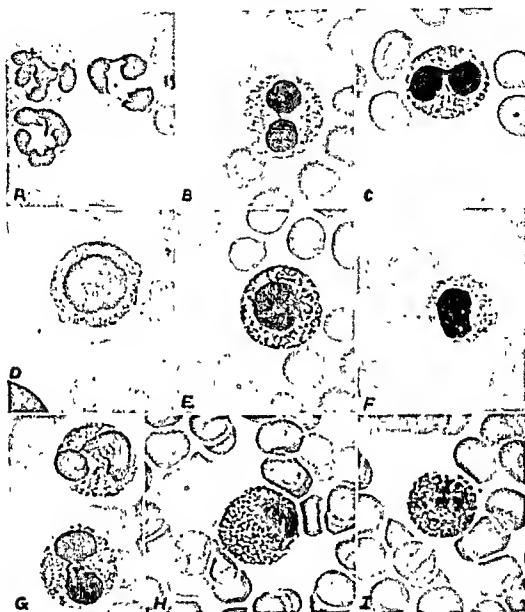


Fig. 42-2 The morphologic changes of Pelger-Huët cells as compared to normal leukocytes. A, Normal neutrophils. B and C neutrophils with bilobed or "pince-nez" nuclei. D, normal myelocyte to contrast with E and F mature Pelger-Huët neutrophils with round or indented nuclei. G, normal eosinophils. H and I mature eosinophils with round nuclei (From Skendzel and Hoffman,<sup>157</sup> courtesy of the authors and Williams & Wilkins Company)

monocytes. Rod-like, dumbbell, peanut-shaped, and spectacle-like ("pince-nez") nuclei with smooth, round, or oval individual lobes (Fig. 42-2, B and C) contrast with the irregular lobes seen in normal neutrophils (Fig. 42-2, A). The incidence of this disorder ranges from as high as 1 in 1000 per-

sons<sup>141,149</sup> to one in 4000<sup>146,157</sup> or 6000,<sup>144</sup> or even one in 10,000.<sup>153</sup> Originally observed mainly in Holland, Germany, and Switzerland, the anomaly has now been described in other parts of the world and in Orientals<sup>160</sup> and Negroes as well as in Caucasians. The practical importance of identifying the Pel-

Table 42-1. Distribution of Nuclear Lobes in Neutrophils of Normal Persons and in Those with Pelger-Huët Anomaly

	Cases Examined	Number of Lobes*				
		1	2	3	4	5
Normals†	50	2.8 ±2.8	22.0 ±6.3	54.3 ±5.3	18.1 ±6.9	2.8 ±2.1
Pelger-Huët heterozygotes†	34	31.3 ±9.2	63.8 9.5	4.9 3.7	0.3 —	0 —
Pelger-Huët homozygotes <sup>141,159</sup>	2	100	—	—	—	—

\*Mean and variance

†Modified from Davidson<sup>144</sup>

ger-Huët anomaly lies in distinguishing this defect from the "shift to the left" that occurs in infection.

The discovery of this anomaly in rabbits led to breeding experiments and the production of homozygotes.<sup>141,157</sup> These studies demonstrated that, in the heterozygote, bilobed, rod-shaped and spectacle forms predominate while, in the homozygote, round nuclei with no evidence of segmentation are predominant. In rabbits the homozygous form was often lethal with most animals dying in utero; the survivors not infrequently suffered skeletal malformations.

Two human homozygotes have been reported.<sup>141,159</sup> In them the cytoplasm of the neutrophils appeared mature, but the nuclei were round or oval in all the neutrophils, in contrast to the fewer than 40% single-lobed neutrophils present in heterozygotes (Table 42-1).<sup>141,144,159</sup> In the homozygotes the eosinophils, basophils, and megakaryocytes also showed dense nuclear chromatin and rounded nuclear lobes; the nuclear lobes were fewer in number than in normal subjects.<sup>141</sup> Examination of the bone marrow revealed normal morphologic features in myeloid precursors through to the myelocyte stage, while electron microscopy revealed persistence of nucleoli in the otherwise mature neutrophils that contained single oval nuclei.<sup>159</sup> This was interpreted as indicating some retardation of nuclear maturation since no cytochemical defects were noted in the cytoplasm.

Pelger-Huët cells appear to be normal functionally,<sup>159</sup> are able to phagocytize mi-

croorganisms,<sup>157</sup> and survive normally in the circulation in both man<sup>154</sup> and the dog.<sup>142</sup>

The Pelger-Huët heterozygote is recognized by finding: (1) 69 to 93% of the neutrophils to be of the bilobed, "pince-nez" type; (2) very few cells with three lobes (usually fewer than 10%); and (3) rare or no cells with four lobes (Table 42-1).<sup>144,146,157</sup> This is in contrast to the findings in normal blood smears in which no more than 27% of the cells are bilobed and significant numbers of cells have three or more lobes (Table 42-1).<sup>157</sup> The presence of similar abnormalities in the blood smear in other family members also is helpful in establishing the diagnosis. In heterozygotes, mature neutrophils with round or oval nuclei of the type that is characteristic of the homozygous state may increase after stresses such as the injection of colchicine<sup>148</sup> or pyripher.<sup>149</sup> A shift towards increased numbers of neutrophil lobes was described in a patient with the anomaly who developed pernicious anemia.<sup>140</sup>

#### Pseudo- or Acquired Pelger-Huët Anomaly

Cells with morphologic changes like those described above have been noted occasionally in association with myxedema, acute enteritis, agranulocytosis, multiple myeloma, malaria, leukemoid reactions secondary to metastases to the bone marrow,<sup>155</sup> drug sensitivity,<sup>150</sup> or chronic lymphocytic leukemia.<sup>149</sup> More commonly, pseudo-Pelger-Huët cells (Plate XVI, C, D, page 1322) are seen in patients with

myeloid leukemia of either the acute or chronic type or in those with myeloid metaplasia.<sup>145,156</sup> In these subjects the pseudo-Pelger-Huet cells tend to appear late in the disease, often, but not always, after considerable chemotherapy has been administered. In addition, the majority of the nuclei are of the single oval type characteristic of the homozygous state.<sup>145</sup>

### Alder-Reilly Anomaly

This anomaly, inherited as a recessive trait,<sup>165</sup> apparently does not interfere with leukocyte function.<sup>173</sup> It is characterized by the presence of larger than normal azurophilic, and basophils ("Alder-Reilly bodies"), which may be easily confused with granulations due to toxic states (Plate XVI, F, G). These granules stain a dark lilac color with Wright-Giemsa stains and have been seen in patients with various types of bone and cartilage abnormalities.<sup>167,176,178,182</sup> However, they are most commonly seen in association with Hurler's syndrome, Hunter's syndrome, and Maroteaux-Lamy polydystrophic dwarfism.<sup>171</sup>

Similar inclusions may be seen in blood lymphocytes ("Gasser's cells") and in blood monocytes.<sup>168,178</sup> The lymphocyte inclusions stain dark red or purple with May-Grunwald-Giemsa stain and metachromatically with toluidine blue, while normal azurophilic granules do not stain at all.<sup>178</sup> Such lymphocyte granules are found in all types of mucopolysaccharidoses except Morquio's syndrome, but they are most frequent in the Hurler, Hunter, Sanfilippo, and Maroteaux-Lamy syndromes.<sup>171,178</sup> They tend to occur in clusters rather than diffusely throughout the cytoplasm, are surrounded by vacuoles, and are shaped like a dot or comma.<sup>178</sup> In one series of 19 patients, from 8 to 50% of the lymphocytes contained the inclusions, and their presence was thought to be of diagnostic significance.<sup>178</sup>

These inclusions are seen inconstantly in the blood, but are more common in the bone marrow. For example, of a series of 18 pa-

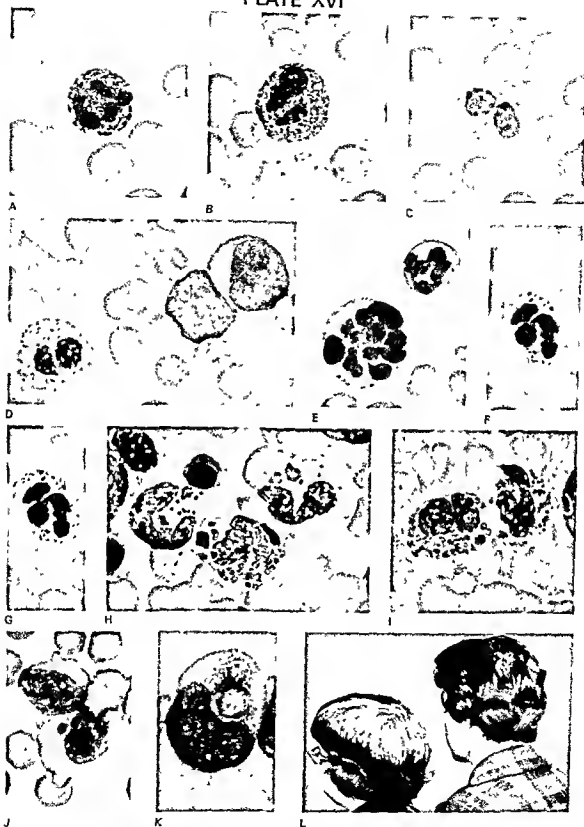
tients with Hurler's form of mucopolysaccharidosis, Alder-Reilly bodies were present in the blood of less than 10%. However, careful examination of the bone marrow revealed mucopolysaccharide granules in large mononuclear cells ("Bukot's cells") in 17 of the 18 patients.<sup>170,181</sup>

The type of inclusion seen is not diagnostic of a particular type of mucopolysaccharidosis; neither is the frequency of the inclusions correlated with clinical severity.<sup>174</sup> It is now clear that the basic defect in this group of diseases lies in the incomplete degradation of the protein-carbohydrate complexes known as mucopolysaccharides, and in the different forms of mucopolysaccharidosis there appear to be different enzymatic deficiencies.<sup>171</sup> The accumulation of partially degraded mucopolysaccharide within lysosomes has been demonstrated by electron microscopy<sup>171</sup>; the degradation of the protein core of the mucopolysaccharide appears to proceed normally, but catabolism of the carbohydrate (glycan) branches is impaired.

### May-Hegglin Anomaly

The May-Hegglin anomaly is a rare, dominantly inherited disorder characterized by large (2 to 5  $\mu$ m), well-defined, basophilic and pyroninophilic inclusions in granulocytes (neutrophils, eosinophils, basophils, monocytes) and accompanied by variable thrombocytopenia and giant platelets containing few granules.<sup>190</sup> For the most part, affected family members have not been ill, but occasionally abnormal bleeding has occurred.<sup>194,199,200</sup> Clot retraction time is prolonged and the reaction to the tourniquet test may be positive. Platelet survival was short (1½ three days as compared with the normal,  $6.9 \pm 1.5$  (1 SD) days).<sup>193</sup> Platelet aggregation and retraction were found to be normal, but spreading and serotonin uptake were increased.<sup>197</sup> Enzyme and substrate content per platelet was increased, but was decreased as related to platelet volume.<sup>197</sup> The granulocyte inclusions (Fig. 42-3) are similar to Dohle bodies (page 1279) in appearance, but often are larger, more round and discrete, and may

# PLATE XVI



Abnormal forms of leukocytes in blood and bone marrow (Wright's stain,  $\times 1000$ ). A and B, Toxic granulation. C and D, Pseudo-Pelger-Huet cells, the latter from the blood of a patient with acute myeloblastic leukemia. E, Hypersegmented polymorphonuclear leukocyte from a patient with myelofibrosis. F, G, Alder-Reilly bodies. H, I, J, K, Chediak-Higashi anomaly (bone marrow) showing inclusions in neutrophils and eosinophils in H and I, in a lymphocyte in J, and in a monocyctoid cell in K. In L, an affected child shows the characteristic silver-gray hair, contrasting with that of her mother. (Courtesy of Dr Dorothy Windhorst, National Institutes of Health.)

formed in most granule-containing cells throughout the body.<sup>213</sup> The resulting abnormalities can be found in the hematopoietic tissues, hair, ocular pigment, skin, adrenal glands, pituitary gland, gastrointestinal organs, peripheral nerves, and elsewhere.<sup>213</sup>

In *neutrophils* the anomalously large, peroxidase-positive granules seen by light microscopy<sup>209,219</sup> have been shown by electron microscopy to be abnormal primary (azurophilic) granules, the contents of which remain pleomorphic, the normal granule crystalloid structure not being formed.<sup>213,217</sup> The specific granules are normal.<sup>213</sup> There appears to be an increased tendency to autophagic vacuole formation in Chediak-Higashi neutrophils, perhaps due to increased permeability and leakage of injurious materials from the massive granules.<sup>235</sup> There is only one type of granule in normal eosinophils and in Chediak-Higashi individuals these are abnormal also.<sup>213</sup> Chediak-Higashi granules also have been demonstrated in mononuclear cells, but their mode of formation is not well understood. In lymphocytes and plasma cells they may be primary granules, but in mononuclear phagocytes and lymphocytes they may be phagolysosomes.<sup>235</sup> Abnormal granules have been observed less frequently in erythroid cells, while in megakaryocytes and platelets the granules appear to be normal.<sup>213</sup>

Similar large granules containing a glycolipid have been observed in the Schwann cells of peripheral nerves, in neurons in the central nervous system,<sup>233,237</sup> in renal tubular cells,<sup>237</sup> and in the vascular endothelium and fibroblasts.<sup>205</sup> In addition, giant pigment granules have been demonstrated in melanosomes<sup>237</sup> and in hair strands.<sup>205,237</sup> In the several different tissues affected, the consistent feature has been that the histochemical reactions of the large abnormal granules are those usually seen in normal granules of that cell line.<sup>237</sup> Since there appears to be no alteration in granule enzyme content, the defect may be in the structure of the lysosome wall. In any case, the effects of the abnormality in different tissues depend on granule function in that tissue. Thus, the large but fewer melanin granules pro-

duce "pigment dilution" and this explains the peculiar hair color, partial albinism, photophobia, and nystagmus.<sup>237</sup> On the other hand, the abnormal, large granules in neutrophils (Plate XVI, H) lead to increased susceptibility to infection. Infection occurs in spite of an above-normal rate of phagocytosis and a normal postphagocytic metabolic burst ( $H_2O_2$  production, etc.).<sup>229</sup> Apparently the intracellular destruction of some bacteria by Chediak-Higashi leukocytes is delayed because the postphagocytic delivery of lysosomal enzymes into phagosomes is inefficient and incomplete.<sup>233</sup> In addition, a defect in cellular response to chemotactic stimuli both *in vitro* and *in vivo* in skin windows has been demonstrated in man and in the mink.<sup>210,211</sup> Finally, in the late stages of the disease, leukopenia and inadequate granulocyte reserves may decrease resistance to infection.<sup>206</sup> The mechanism for this may relate to a postulated destruction of granulocytes within the marrow<sup>206</sup> or to the effects of the large spleen, or because of marrow infiltration by mononuclear cells during the accelerated phase.

**INHERITANCE.** Fifty-six affected children have been reported in families with a total of 127 children.<sup>205</sup> Judging by this and on the basis of animal studies,<sup>228</sup> this disorder is almost certainly inherited as an autosomal recessive trait. Males and females have been affected in a ratio of 0.87:1. A high proportion of marriages producing affected children have been consanguineous. Some heterozygotes may be identifiable by the presence of granulation in some of their lymphocytes.<sup>215,226</sup>

**CLINICAL FEATURES AND COURSE.** As already mentioned, the partial albinism (more properly, pigment dilutional defect<sup>237</sup>), "silvery" hair (Plate XVI, L), and photophobia are usually noted early in infancy. The poor resistance to respiratory and cutaneous infection, especially by staphylococci and other gram-positive organisms, soon becomes evident. In four patients studied for more than one year there were 29 episodes of fever and pyogenic infection.<sup>240</sup> Many of the afflicted children die of infection during infancy or



early childhood. In others the disease remains quiescent or changes to an "accelerated" phase characterized by lymphadenopathy, hepatosplenomegaly, neuropathy, anemia and neutropenia (90%), and less often thrombocytopenia (67%). During this phase there is widespread infiltration of the tissues by mononuclear cells; this has been termed "lymphoma" by some, but it is more likely a reactive lymphohistiocytic response.<sup>205,222</sup> During the accelerated phase, neurologic manifestations (peripheral neuropathy) may become prominent and hemorrhage may occur.

**LABORATORY FINDINGS.** The characteristic microscopic findings are the large, often multiple, peroxidase-positive lysosomal granules in the granulocytes of the blood and bone marrow, and the large melanosomes in the hair. Less frequent are granules in the lymphocytes. During the early phases of the disease, blood counts give normal values, but as the disease progresses anemia, neutropenia, and thrombocytopenia frequently develop. Immunoglobulin and complement levels are normal as are cellular immune reactions. Later, in the accelerated phase, erythrocyte and granulocyte survival may be shortened.<sup>205</sup>

**MANAGEMENT.** The infections, when they occur, are managed according to generally accepted medical principles. The prophylactic administration of antibiotics has not proved to be beneficial.<sup>240</sup> In the accelerated phase, splenectomy has been only temporarily helpful<sup>205</sup>; best results have been obtained with a combination of vincristine and prednisone therapy, but only a few patients have been so treated.

#### Abnormal Specific (Secondary) Granule Formation

A syndrome of lifelong recurrent staphylococcal skin and sinus infections associated with abnormal chemotaxis, impaired staphylococcal killing, and morphologic abnormalities in the neutrophils was described in a 14 year old boy.<sup>250</sup> No other family members

were affected. The patient's polymorphonuclear neutrophils exhibited bilobed nuclei with unevenly distributed chromatin, drumstick-like nuclear projections, and nearly absent cytoplasmic granules that stained with peroxidase but not with alkaline phosphatase. On electron microscopy, primary granules were present, but specific granules were small and reduced in number. These neutrophils were capable of phagocytosis, generated  $H_2O_2$ , reduced NBT dye, and killed *Candida*, but staphylococcal killing was impaired.

#### Familial Vacuolization of Leukocytes (Jordan's Anomaly)

This disorder is characterized by the presence of vacuoles in the cytoplasm of granulocytes, monocytes, and occasionally in lymphocytes and plasma cells. In members of one family, all of the blood neutrophils and more than 70% of the monocytes contained 3 to 10 vacuoles ranging in size from 2 to 5  $\mu m$ , while fewer and smaller vacuoles were seen in eosinophils, basophils, and lymphocytes.<sup>246</sup> By histochemistry and fluorescence microscopy the vacuoles were shown to contain lipids. These were seen in promyelocytes, myelocytes, metamyelocytes, and occasionally in plasma cells in the bone marrow; they were not present in myeloblasts, erythroblasts, or megakaryocytes.<sup>246</sup> The disorder appears to be familial. Two members were affected in each of two unrelated families.<sup>245,246</sup> There was no acute disease in any of the four patients, but, in members of one of the families, progressive muscular dystrophy was present,<sup>246</sup> while, in members of the other, ichthyosis was associated.<sup>245</sup> This type of vacuolization must be distinguished from that characterized by fat-staining vacuoles occurring in persons with serious infections, toxic hepatitis, or diabetic ketoacidosis<sup>245</sup> (also see Chapter 41, page 1278).

#### Other Inclusions in Leukocytes

In an infant with congenital bile duct atresia, amorphous, round to oval bodies that stained green or gray-green with Romanow-

sky stains were seen in 3 to 13% of the blood neutrophils and in 1 to 5% of the monocytes.<sup>218</sup> Similar inclusions were present in all stages of myeloid cells in the bone marrow but not in lymphocytes or plasma cells. Electron microscopy showed that the inclusions were not enclosed in a phagocytic vesicle.

In the blood monocytes of patients with the Hermansky-Pudlak syndrome,<sup>251</sup> a rare familial disorder characterized by albinism, mild bleeding due to platelet dysfunction, and accumulation of ceroid-like pigment in marrow macrophages, lipopigment bodies as well as another type of inclusion were demonstrated.

### Hereditary Giant Neutrophilia

Neutrophils with a diameter of about 17  $\mu\text{m}$  (as compared to a normal diameter of about 13  $\mu\text{m}$ ) are rare in blood smears from normal people (one in 20,000 neutrophils or less). They may be seen with greater frequency in patients who are ill, but even then the number rarely exceeds 0.2% unless a disease involving leukocyte production is present or a reaction to a cytotoxic drug occurs.<sup>216</sup> A family with giant neutrophils in healthy members of three generations has been reported.<sup>255</sup> Over several years the propositus had an average of 1.6% giant neutrophils in his blood. The large neutrophils appeared to be nearly double the normal cell volume and contained from 6 to 10 nuclear lobes. Because of this it was suggested that the cells may have been tetraploid. This anomaly appeared to be transmitted as an autosomal dominant trait.

### Hereditary Hypersegmentation of Neutrophil Nuclei

Several families whose members had a hereditary (autosomal dominant) increase in the number of neutrophil nuclear segments have been described.<sup>262</sup> The proportion of neutrophils containing five lobes or more exceeded 10% in most heterozygotes and was greater than 14% in several suspected homozygotes, as compared to no more than 10% in normal controls.<sup>262</sup> The bone marrow

findings suggested a tendency to nuclear indentation in early myeloid forms (eosinophils and basophils as well as neutrophils).<sup>262</sup> The normal size of these neutrophils was thought to provide evidence against tetraploidy, but in one study of five female family members the mean number of nuclear drumsticks appeared to be increased above normal.<sup>260</sup> The chief significance of this anomaly is in its differentiation from other causes of hypersegmentation such as folate or vitamin B<sub>12</sub> deficiency (page 568).

### Hypersegmentation of Eosinophils and Negative Staining for Peroxidase and Phospholipids<sup>272</sup>

This disorder is inherited as an autosomal recessive trait and is characterized by a lack of sudanophilia and peroxidase activity in all of the eosinophils, whereas these histochemical reactions remain positive in the neutrophils and monocytes.<sup>270</sup> In addition, the number of eosinophilic granules per cell appears to be reduced and there may be some hypersegmentation of the eosinophil nucleus. No disease accompanies the disorder and to date it has been reported only in people of Jewish (predominantly Yemenite) extraction. However, members of other races have not been studied adequately.

## Functional Disorders of Leukocytes Not Characterized by Morphologic Changes

### Chronic Granulomatous Disease (CGD) of Childhood<sup>281,297,303</sup>

This disorder represents a lethal, inherited defect of leukocyte function which is associated with no evident morphologic abnormalities. Its exact nature is not yet understood and, indeed, it is likely that several different enzymatic defects result in a similar clinical and pathologic picture (see Variants, page 1328). In affected males there is a history, beginning in early childhood, of recurrent suppurative infections caused by organisms

of low-grade pathogenicity, such as *Serratia marcescens*, enterobacteria (*Klebsiella*, *Aerobacter*, or *Salmonella*), or by staphylococci. The eczematoid, granulomatous and sometimes purulent skin infections recur over and over again and clear slowly. Associated adenopathy develops and may persist, giving a picture of *scrofula*. Pulmonary and other infections (eg, osteomyelitis) also are common and progressive granulomatous disease of the lungs, liver, and other sites develops. Hepatosplenomegaly is common and biopsy reveals necrotizing granulomas, often with associated purulent inflammation. The disorder usually progresses to death in early childhood,<sup>283</sup> but occasional patients have survived into the late teens.<sup>284,312</sup> Although rare, this disorder has assumed great importance as a prototype of defects in leukocyte bactericidal capacity.

**HISTORY AND MODE OF INHERITANCE.** The syndrome was first described in 1957<sup>283</sup> as affecting only males. More than 90 cases have now been reported.<sup>303,312</sup> X-linked inheritance has been established. Female heterozygotes are identifiable by the presence of defective leukocyte bactericidal capacity intermediate in degree between that of affected patients and that of normal individuals.<sup>323</sup> Measurements of postphagocytic release of <sup>14</sup>CO<sub>2</sub> from glucose-1-<sup>14</sup>C or reduction of nitroblue tetrazolium (NBT) dye can also be used to detect the defect. By means of the NBT dye test it was found that in carrier females there is a mixed population of neutrophils, about half being NBT negative and half positive (Table 42-2). The defect appears to be transmitted on an X chromosome and the degree of deficit in females varies according to random X chromosome inactivation. Consequently, one may expect to find an occasional carrier female with clinical disease as severe as that in the males.<sup>294,323</sup>

**ETIOLOGY AND PATHOGENESIS.** Because of the clinical and pathologic picture of chronic recurrent infection caused by low-grade pathogens and the resultant granuloma formation, it was suggested that a defect in the inflammatory response results in lesions

**Table 42-2. Proportion of Postphagocytic Neutrophils That Are NBT Positive in Family Members and Patients with Chronic Granulomatous Disease as Compared to Normals<sup>323</sup>**

	Number	% NBT Positive Cells*
Patients (hemizygotes)	7	9.9 ± 4.2
Mothers & grandmothers (heterozygotes)	9	49.8 ± 5.4
Carrier sisters	7	51.3 ± 6.6
Fathers, brothers, and normal sisters	18	74.4 ± 5.4
Normals	12	89.5 ± 5.4

\*Mean ± 2 SD

comparable to those that normal persons develop following infection with tubercle bacilli or brucella.<sup>297</sup> No abnormality in antibody response to diphtheria, tetanus, or polio virus was demonstrated in these patients, and their delayed hypersensitivity response also was normal.<sup>297</sup> In addition, leukocyte migration and surface phagocytosis were normal.<sup>297</sup> Nevertheless, when leukocytes of CGD patients were incubated with bacteria, such as staphylococci, *Serratia*, or other organisms causing infection in these patients, decreased bacterial killing and prolonged bacterial survival in the phagocytic vacuoles were noted.<sup>307,317</sup> Decreased killing of fungi and incomplete inactivation of vaccinia and herpes virus have also been reported,<sup>297,311</sup> but streptococci are killed normally by CGD leukocytes.<sup>305,317</sup>

Although the mechanisms of bacterial killing by normal leukocytes are incompletely understood, it is clear that phagocytosis is followed by fusion of lysosomes with the phagocytic vacuole, discharge of lysosomal enzymes into the phagosome, and a burst of postphagocytic metabolic activity. These events are usually accompanied by bacterial death and digestion (see page 259, Chapter 6). Initial studies of CGD leukocytes suggested that the defect in bacterial killing might result from decreased degranulation and delivery of lysosomal enzymes into phagocytic vacuoles.<sup>297,317</sup> However, other

studies demonstrated normal lysosomal degranulation.<sup>277,307</sup> In still other experiments, smaller than normal phagosomes without normal enlargement<sup>314</sup> were described. Attempts to demonstrate a deficiency of lysosomal enzymes have been unsuccessful, normal activities of acid phosphatase, beta glucuronidase, peroxidase, lysozyme, and phagocytin having been found.<sup>297,317</sup> A deficiency of cytoplasmic NADH oxidase activity was reported in five patients with CGD and it was suggested<sup>275</sup> that this deficiency might explain the observed defects in post-phagocytic oxygen consumption, oxidation of glucose via the hexose monophosphate shunt (release of  $\text{CO}_2$  from the 1-carbon of glucose), formate oxidation, NBT dye reduction, and bacterial killing.<sup>298-314</sup> On the other hand, others have not found a deficiency of NADH oxidase activity,<sup>299</sup> and it has been reported that a deficiency of NADPH oxidase or an abnormality in its activation is the underlying cause of this syndrome.<sup>297a</sup> In either case, the finding that iodination of bacteria in the phagosome appears to be one mode of bacterial killing, and that this is defective in CGD leukocytes and can be partially repaired by the insertion of an oxidase into the phagosome, either carried in on oxidase-coated polystyrene particles<sup>278,302</sup> or as part of the ingested bacteria,<sup>309</sup> provides strong evidence incriminating defective generation of  $\text{H}_2\text{O}_2$ , superoxide,<sup>247</sup> or related ions<sup>290a</sup> as the pathogenic defect in this disorder.<sup>275,278</sup> Although most studies have involved the neutrophils, it was recognized in the earliest reports that lipid-laden macrophages were present in the granulomatous lesions<sup>310</sup> and a defect in the mononuclear phagocytes<sup>290</sup> and in eosinophils<sup>314</sup> has since been demonstrated. The platelets apparently are normal.<sup>292</sup>

Since the generation of peroxide and bacterial killing may involve several enzymes (myeloperoxidase, NADH or NADPH oxidase, glutathione peroxidase, glutathione reductase, and the enzymes of the hexose monophosphate shunt), one might expect that defects other than NADH or NADPH deficiency would result in a disorder pheno-

typically like that of CGD. Several have already been described (see Variants, below).

**CLINICAL AND LABORATORY FEATURES.** The clinical picture of chronic granulomatous disease, as mentioned earlier, includes the development of recurrent infections, especially of the skin and lungs, with septic lymphadenitis, development of hepatosplenomegaly and granulomas, and ultimately death from infection.<sup>283,284,297</sup>

The blood neutrophil count is not reduced and rises appropriately with infection or after endotoxin injection; monocytosis is sometimes observed.<sup>297</sup> Since chronic infection is common, the immunoglobulin levels often are elevated, and plasma cells may be present in increased numbers in the bone marrow. Immunoglobulin deficiencies have not been encountered and antibody titers (both IgM and IgG) increase normally after antigenic stimulation. Reticuloendothelial clearance of colloidal gold also is normal. Peroxidase staining of blood cells demonstrates more intense dye uptake in CGD neutrophils than in normal cells.<sup>311</sup> The simplest method for detecting the defect is the postphagocytic, intraphagosomal reduction of almost colorless NBT dye to blue-black formazan (Fig. 42-4).<sup>276,314</sup> When properly standardized in regard to time of incubation of leukocytes with the dye-tagged zymosan particles, affected patients and most carriers are readily recognized (Table 42-2). When the reaction to this test is negative in suspected carriers the reaction to the quantitative dye reduction test will usually be positive.<sup>311</sup>

**COURSE AND PROGNOSIS.** This disease runs a progressive downhill course due to repeated infections and granuloma formation. The average life expectancy is five to seven years.<sup>297</sup>

**VARIANTS.** Since the clinical picture of chronic granulomatous disease results from delayed killing of catalase-positive bacteria, enzymatic defects in the bactericidal system other than decreased oxidase (NADH or NADPH) activity (Chapter 6, page 259) may produce an almost identical picture.

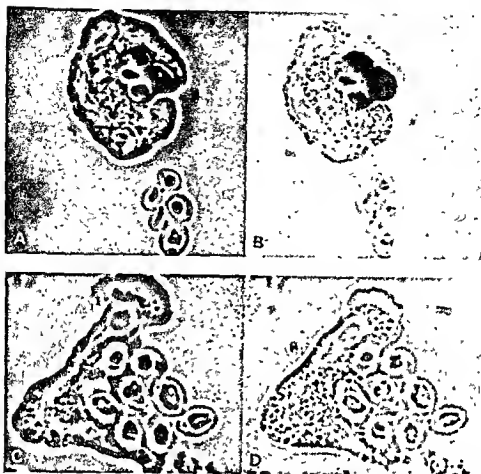


Fig. 42-4 Phase (A and C) and bright field (B and D) photomicrographs of normal neutrophil (A and B) and a neutrophil from a patient with chronic granulomatous disease (C and D). In A and B, note NBT reduction (black area) that has occurred in the normal neutrophil 20 minutes after ingestion of zymosan granules and NBT dye. In C and D zymosan ingestion is normal but there is no dye reduction (From Nathan, Baehner, and Weaver,<sup>314</sup> courtesy of the authors and the Journal of Clinical Investigation)

*Glutathione peroxidase deficiency* was found in two unrelated females, 9 and 13 years old, presenting a clinical and metabolic picture similar to that of chronic granulomatous disease and with decreased bactericidal capacity.<sup>299</sup> The findings in these girls differed from those in males with CGD only in that no heterozygotes were detected in their families and their clinical course was somewhat milder.

*Lipochrome histiocytosis* was described in three sisters with rheumatoid arthritis, hyperglobulinemia, splenomegaly, pulmonary infiltrates, and increased susceptibility to infection.<sup>296</sup> No granulomas were found in tissue biopsies, but lipochrome pigmentation in large macrophages was present throughout the tissues. Studies of blood leukocyte func-

tion in two of the sisters revealed impaired postphagocytic respiration, NBT reduction, and hexose monophosphate shunt activity identical to that seen in chronic granulomatous disease.<sup>318</sup>

A bactericidal defect has also been described in a patient with a complete deficiency of *glucose-6-phosphate dehydrogenase* (G-6-PD)<sup>286</sup> (Chapter 23). Apparently almost complete absence of G-6-PD activity is necessary to interfere with bacterial killing since no difference from normal could be detected in cells with 25% activity or greater.<sup>319</sup>

"*Job's syndrome*" was reported as occurring in two unrelated girls with red hair and fair skin who suffered from repeated staphylococcal cold abscesses, sinusitis, eczema, and pulmonary disease, a syndrome not unlike the

affliction of Job.<sup>288</sup> The leukocytes of these patients were capable of normal phagocytosis, bacterial killing, NBT reduction, and iodine fixation,<sup>289,321</sup> unlike those of patients with CGD. An additional case of Job's syndrome was reported in 1971.<sup>315</sup> Two sisters, the children of parents who are second cousins; also have been reported as having this disease,<sup>281</sup> but they probably did not have it since their leukocytes did not reduce NBT or kill bacteria normally.

Hydrocortisone produces a defect in bacterial killing by neutrophils that is similar to that seen in chronic granulomatous disease.<sup>313</sup>

### Myeloperoxidase Deficiency

An inherited (probably autosomal recessive) deficiency of myeloperoxidase (MPO) in the neutrophils and monocytes, but not the eosinophils, has been described.<sup>331</sup> The 49 year old male propositus, one of his two sisters, and all four sons exhibited decreased MPO activity, but no increased frequency of infections accompanied this defect. Also in two other families apparently similarly affected, infection was not a problem.<sup>334</sup> These findings indicate that mechanisms other than MPO-catalyzed bacterial killing are functionally important in human neutrophils.<sup>331</sup>

A similar but acquired defect in MPO activity was reported in a patient with myelomonocytic leukemia<sup>330</sup> and in another with a refractory anemia.<sup>335</sup>

### Other Enzymatic Defects

Two siblings with negative peroxidase, oxidase, and lipid reactions in the neutrophils and monocytes also have been reported.<sup>332</sup>

## Disorders Involving the Monocyte-Macrophage System

The abnormalities to be discussed here include the familial sphingolipidoses and those conditions, sometimes acquired, that may simulate them in that "storage" cells

may be found in the bone marrow, blood, or other tissues. Most of these disorders are the result of inherited enzymatic defects. Thus they are not, strictly speaking, diseases of the monocyte-macrophage system. However, their major manifestations often result from accumulation of incompletely catabolized tissue products in the cells of this system and therefore it seems appropriate to include them here. They are of interest to the hematologist since, in some of them (eg, Gaucher's disease, Niemann-Pick disease), anemia, leukopenia, thrombocytopenia, or hepatosplenomegaly may be the mode of presentation. In others (sea-blue histiocyte syndrome, lipochrome histiocytosis) the presence of pigment-containing cells in the bone marrow, lungs, or other sites, or skin lesions simulating petechiae (Fabry's disease, Fig. 42-10A, page 1341) may bring them to his attention.

Still other disorders such as Tay-Sachs disease, although closely related biochemically (Fig. 42-6B), primarily involve the nervous system and the hematologist is seldom consulted.

**PATHOGENESIS.** The fundamental defect in all of these lipidoses is the accumulation of ceramide compounds in various cells and tissues. Ceramide is an acylated sphingosine (Fig. 42-5A and B) and is the backbone of a variety of compounds, called sphingolipids, that have major structural functions in many cells. The fatty acid portion of sphingolipid differs in various tissues, being mainly stearic acid ( $C_{18}$ ) in the brain and somewhat longer fatty acids ( $C_{20}$  to  $C_{24}$ ) in non-neural tissue. The distinguishing feature and function of each sphingolipid are determined by the compound(s) esterified to the number one-carbon of ceramide (Figs. 42-5A and 42-6). For example, the addition of hexoses and *n*-acetylneuraminic acid to ceramide forms the group of compounds known as gangliosides that are found chiefly in the brain (Fig. 42-6B). Other ceramide compounds, the globosides (Fig. 42-6A), are found in cell membranes, including those of erythrocytes, leukocytes, and platelets, where they function as haptens and may be important in defining the immunochemical specificity of the cell surface.<sup>343</sup>

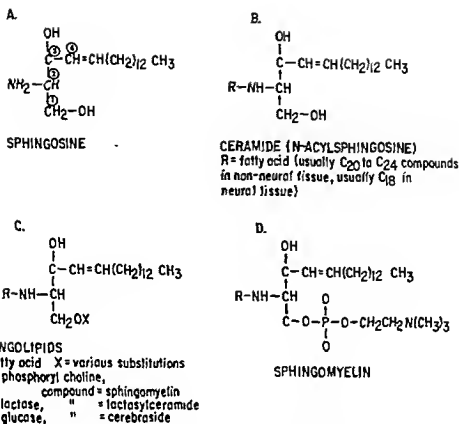


Fig 42-5. Formulae of some of the sphingolipids

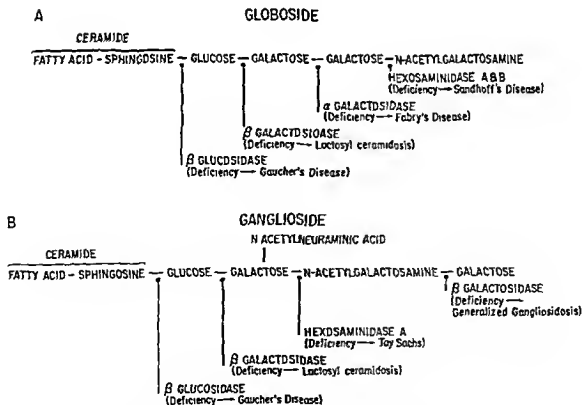
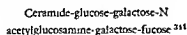


Fig. 42-6 Schematic structure of globoside and ganglioside to show site of action of the several catabolic enzymes, which, when defective, result in one of the storage diseases

Blood group antigens also are ceramide compounds; thus, H-isoantigen is:



Theoretically these lipids may accumulate as a result of: (1) increased synthesis within cells, (2) increased uptake from outside sources; (3) a chemical defect in the lipid that interferes with catabolism, (4) a defect in lipid catabolic enzymes; or (5) combinations of these mechanisms. In the hereditary lipidoses, studies have revealed no evidence for overproduction of lipid,<sup>421</sup> but clear-cut deficiencies in specific catabolic enzymes have been well documented.<sup>310,311</sup> On the other hand, in some of the acquired disorders no enzymatic defects have been demonstrated and an overloading of normal lipid catabolic mechanisms seems likely (see page 1336). Regardless of the basic mechanism, one would expect that the tissues most seriously affected would be those in which lipid turnover is high, either because of normal metabolic requirements, as in the developing nervous system, or where lipid catabolism is a major cell function as a result of phagocytosis and scavenging activity, as in the monocyte-macrophage system. Thus, indeed, appears to be the case.

### Gaucher's Disease

**DEFINITION AND HISTORY.** Gaucher's disease is a rare, chronic, familial disorder characterized clinically by hepatosplenomegaly,

skin pigmentation, pingueculae of the sclerae (Fig. 42-7), bone lesions, and, in its later stages, anemia, leukopenia, or thrombocytopenia. Histologically, large cerebroside-containing cells (Fig. 42-8 and Plate II, G, p. 72) are found, particularly in the spleen but also in the bone marrow, liver, and elsewhere.<sup>371</sup> The condition was described by Gaucher in 1882<sup>383</sup> and the presence of glucocerebroside in the cells, first recognized by Epstein<sup>381</sup> and by Lieb<sup>403</sup> in 1924, has since been amply confirmed.<sup>415</sup> That the metabolic defect in Gaucher's disease is deficient activity of a catabolic enzyme,  $\beta$ -glucocerebrosidase, was established by Brady and associates (Fig. 42-6).<sup>369</sup>

**ETIOLOGY AND PATHOGENESIS.** Gaucher's disease is most often discovered during childhood. A considerable proportion of the patients have been Jews, especially those from the Baltic Sea area, but the condition has been found in non-Jewish Caucasians and in natives of Greece,<sup>375</sup> India, China, and Japan; it has also been reported in Negroes.<sup>371,390,393</sup> Commonly, several cases are found in a family,<sup>374</sup> but it is unusual for more than one generation to be affected. There is no preference for either sex. From the more than 1000 cases of Gaucher's disease reported, it has been inferred that the mode of inheritance is autosomal recessive.<sup>345</sup> This was substantiated by cell culture techniques whereby tissues from patients with Gaucher's disease were shown to have markedly decreased  $\beta$ -glucosidase activity.<sup>347,364</sup>



Fig 42-7. Pingueculae in a patient with Gaucher's disease





Fig 42-8. Typical Gaucher cell together with a lymphocyte and a juvenile neutrophil from the sternal bone marrow in a patient with Gaucher's disease

The enzyme activity in carriers was intermediate between the normal level and that found in affected patients.<sup>347,394,395</sup> Some family studies, however, have suggested occasional dominant transmission.<sup>390,398</sup> It has been proposed that this may have resulted from marriage between an affected person and a heterozygote.<sup>345,399</sup> The assay of  $\beta$ -glucosidase activity in such kindreds should reveal whether adult Gaucher's disease is ever transmitted as an autosomal dominant trait.

Two different  $\beta$ -glucosidase activities, possibly representing different isozymes, with different pH optima (4.0 and 5.3) have been found in leukocytes from adults with Gaucher's disease.<sup>364,395</sup> However, in skin fibroblast cultures from these patients only one enzymatic activity was detected.<sup>364</sup>

As a result of deficiency in  $\beta$ -glucocerebrosidase activity,<sup>369</sup> cleavage of glucose from ceramide is impaired (Fig. 42-6) and glucose-ceramide accumulates within cells where it is apparently is largely confined within lysosomal membranes.<sup>350</sup>

In the adult form of Gaucher's disease the major source of the accumulated glucocerebroside in the tissues appears to be senescent leukocytes.<sup>340,400</sup> Compared with erythrocytes, neutrophils contain about 400 times as much glycolipid per cell, chiefly as ceramide dihexoside.<sup>343</sup> From daily cell turnover rates it has been calculated that granulocyte turnover requires the synthesis and catabolism of nearly 400 mg ceramide lactoside per day, 20 to 40 times as much as is involved in erythrocyte turnover per day.<sup>340</sup>

In patients with the infantile form, glucocerebrosidase activity in the tissues is very low (0 to 9% of normal) in contrast to the adult form in which activity is 12 to 44%.<sup>341</sup> In the infantile form, the central nervous system is the major site of involvement. It is not yet clear whether or not the accumulation of lipid in neurons is followed by cell degeneration, phagocytosis, and transport of these lipids to the spleen, liver, and elsewhere by macrophages, but such a process may at least add to the other sources of glycolipid in the reticuloendothelial system.

**SYMPTOMATOLOGY.** In general, the onset of Gaucher's disease is earliest and progression is most rapid in patients with the least glucocerebrosidase activity. The enzyme level and clinical picture have been found to be relatively constant in a given family, thus suggesting that the disorder is genetically heterogeneous; perhaps several different mutations affect the same or similar loci and alter the composition of the enzyme.<sup>364</sup>

Gaucher's disease has been divided into three forms—infantile, juvenile, and adult—based on the time of appearance of clinical symptoms,<sup>367,409</sup> but some do not differentiate the juvenile and adult forms since they appear to differ mainly in their rate of progression.<sup>341</sup>

The adult type is by far the most common, more than 900 cases having been reported.<sup>345</sup> The term "adult" is somewhat of a misnomer because symptoms usually begin in childhood or early adulthood. Physical development often is normal. Splenomegaly usually is the outstanding sign.<sup>406</sup> It may be discovered

accidentally or because of abdominal fullness; more rarely the weight of the large spleen produces a dragging sensation, or infarction with sharp pain may develop. The liver usually is also enlarged, but there is little enlargement of the superficial lymph nodes. Infrequently, splenomegaly is inconspicuous or absent, and anemia, thrombocytopenia, bone lesions, or other findings lead to the diagnosis.<sup>365,406 409 414</sup> Ascites is rare.<sup>399</sup> Hemorrhage, especially from the nose and gums, is relatively common and occasionally petechiae or purpuric spots are noted.

Skin pigmentation, ochre to brown in color with a yellow or leaden hue, was found in 45 to 75% of patients in some series.<sup>367</sup> The head, neck, and hands were affected and symmetrical pigmentation of the legs from just below the knees to the instep was described.<sup>367</sup> In other reports, however, skin pigmentation and brown pingueculae (Fig. 42-7) were described only in a minority of patients and were said to appear when the disease was at an advanced stage.<sup>406</sup> The pingueculae contain the typical Gaucher cells.<sup>380</sup>

The accumulation of Gaucher cells in the bones may be associated with macroscopic areas of destruction. The cells may be found in the femur, hip bones, vertebrae, humerus, or tibia<sup>381 416</sup> and are seen roentgenographically in about 75% of the patients. The femur is most commonly involved, especially its head and neck; the swelling in its lower end often resembles an Erlenmeyer flask in shape.<sup>419</sup> The bone may be increased in diameter and the cortical layer thinned<sup>367</sup> or there may be areas of rarefaction and condensation. Although only 50% of patients with bone lesions have symptoms referable to the bones,<sup>419</sup> severe pain in the limbs,<sup>408,409</sup> crippling,<sup>406</sup> and collapse of vertebral bodies with gibbus or pathologic fractures may occur.<sup>408,414</sup>

A much less common form of Gaucher's disease is that seen in *infants*. This is characterized by retarded development, early onset of neurologic signs (convergent strabismus, dysphagia, opisthotonos, and multiple signs of brain stem involvement), hepatosplenomegaly,<sup>341,345,347</sup> cachexia, and death—

usually before the age of 2 years. Bone involvement is rarely, if ever, present in these patients.<sup>388</sup> This form also is transmitted as an autosomal recessive trait and often is found in several members of a family. Consanguinity is not uncommon. The infantile type of the disease rarely occurs in Jews.<sup>369,406</sup>

The third, somewhat heterogeneous type (*juvenile Gaucher's disease*) was reported in several interrelated families in Sweden.<sup>394</sup> The onset took place when the patient was six months to a year of age, but cerebral signs appeared later than in the infantile type and 6 of the 12 patients survived into late childhood or the teens.

**THE BLOOD.** The *anemia* is usually moderate in degree and normocytic in type. There is little or no evidence of active blood regeneration such as polychromatophilia or nucleated red cells. In one patient with massive splenomegaly, the red cell mass was normal but the plasma volume was increased, thus it was inferred that the anemia was due to hemodilution.<sup>368</sup> In four other patients a mild decrease in red cell survival was accompanied by decreased incorporation of iron into red cells, and it was concluded that the anemia resulted from a moderate degree of ineffective erythropoiesis.<sup>402</sup> In addition, there undoubtedly is considerable red cell sequestration in the large spleen. *Leukopenia* is common but is of little clinical importance since resistance to infection does not seem to be impaired.<sup>406</sup> *Thrombocytopenia* is present in the majority of patients and can be the most troublesome hematologic manifestation.<sup>406</sup> It is usually mild with platelet counts above  $70.0 \times 10^9/l$ , but sometimes severe thrombocytopenia with bleeding may develop.<sup>376,389</sup> In one such patient the platelet half-disappearance time was reduced to 0.8 days and there was marked sequestration of labeled platelets in the spleen. After splenectomy the  $t_{1/2}$  increased to five days and the platelet count rose to normal levels.<sup>386</sup> Gaucher's cells have been demonstrated in the blood, but this usually requires careful examination of buffy coat preparations.<sup>366,385</sup>

The *serum acid phosphatase* activity, when

measured with phenyl phosphate as substrate, usually is increased.<sup>406,421,425</sup> Several isozymes have been detected; some apparently are the same as those found in normal spleen and platelet lysosomes.<sup>421</sup> In contrast to the prostatic enzyme, the acid phosphatase in the serum of patients with Gaucher's disease is less inhibited by 1-tartrate.<sup>406,421</sup> The cytoplasm of Gaucher's cells shows strong acid phosphatase activity.<sup>373,417</sup>

Monoclonal immunoglobulin peaks have been described in a number of patients with adult Gaucher's disease, but their significance remains obscure.<sup>413</sup>

**DIAGNOSIS—THE GAUCHER CELL.** Massive splenomegaly associated with no evidence of cachexia, particularly if accompanied by skin pigmentation, brown pingueculae, bone pain, a tendency to easy bruising, and moderate thrombocytopenia suggests Gaucher's disease. The diagnosis depends on finding the typical Gaucher's cells (Fig. 42-8 and Plate II, G) in biopsied tissue. The cells are present in the bone marrow, but splenic or liver aspirates, lymph nodes, and the kidneys also contain these cells.<sup>371</sup>

In marrow aspirates the cells are most easily detected in the thicker portions of the smear by scanning with a low-power objective. The large diameter (20 to 80  $\mu$ m), pale blue, engorged histiocytes are usually easily found and can then be more carefully examined under higher-power magnification (Plate II, G). They are usually round or oval and may possess one or several eccentrically placed nuclei. The cytoplasm appears faintly blue in Wright's-stained smears or in smears stained with Mallory's aniline blue, and numerous fibrillae can be seen. The cells also stain faintly with eosin and are PAS and Sudan black B positive.<sup>359</sup>

**TREATMENT.** Treatment of patients with the adult form of Gaucher's disease is purely symptomatic. Splenectomy may be considered if the weight of the spleen or splenic infarcts become a problem, but the major indication for splenectomy in these patients is severe thrombocytopenia with bleeding.<sup>386,404</sup> The appearance of Gaucher's cells in extrasplenic organs such as the bones and

liver may be accelerated following this operation.<sup>406,427</sup>

Patients with manifest Gaucher's disease, with rare exceptions,<sup>398</sup> produce only healthy offspring, half of whom will be carriers. Those who already have produced a child with the disease have a 50% chance of producing additional affected offspring.<sup>390</sup> Culture of amniotic fluid cells and assay for  $\beta$ -glucosidase activity permit detection of affected fetuses in families at risk.<sup>341</sup>

Since human and beef spleen preparations of purified exogenous glucocerebrosidase are now available,<sup>340</sup> their administration to patients with very low enzyme levels offers hope for specific therapy. It is reasonable to believe that administered enzyme will be taken up by the reticuloendothelial cells. That the enzyme will be taken up by the central nervous system seems less likely.

Splenic transplantation has been attempted in end-stage, juvenile Gaucher's disease, but the graft functioned for only about 40 days and the patient died three months post-transplantation.<sup>391</sup>

**PROGNOSIS.** Patients with the adult form of Gaucher's disease may live long and successful lives if disease progression is slow, and some have survived to old age.<sup>390</sup> A number have survived pregnancy without difficulty.<sup>397</sup> Long survivals (15 to 30 years) after splenectomy have been recorded.<sup>401</sup> In patients with the infantile form, however, death usually occurs in early childhood. In those with the juvenile form the rate of progression is more rapid than in adult onset disease and the prognosis is particularly poor if there is neurologic involvement.

**PATHOLOGY.**<sup>350</sup> The pathognomonic feature of Gaucher's disease is the infiltration of tissues by Gaucher cells.<sup>377</sup> Under the electron microscope these cells are seen to be filled with numerous, elongated, rod-shaped bodies that contain smooth-walled tubular elements and react with acid phosphatase.<sup>412</sup> They presumably are secondary phagosomes.

In the chronic form of the disease the splenic pulp is densely infiltrated with Gaucher cells. In the liver the cells are found mainly in the centrilobular areas, the Kupffer cells

usually appear to be normal.<sup>350</sup> In the bone marrow the Gaucher cells are often found in large groups and in the lymph nodes they are associated with the reticulum. The spleen often is increased to ten times normal size; weights in excess of 6000 g have been recorded. The liver may be twice normal size.

In the infantile form the lungs, kidneys, adrenals, ovaries, pituitary gland, thymus gland, and brain also are involved. There is acute neuronal degeneration,<sup>360</sup> but little neuronal storage of lipid is evident.<sup>360</sup> Gaucher bodies are only occasionally seen in neurons.<sup>350 360 410</sup>

**GAUCHER CELLS IN OTHER DISORDERS.** Gaucher cells at first were considered pathognomonic of the inherited disorder described above. More recently they have been detected in marrow specimens of patients with chronic myelocytic leukemia (CML), eg, in 10 out of 64 patients in one series.<sup>362</sup> In a patient with CML with a leukocyte count of  $8000 \times 10^9/l$ , electron microscopy revealed typical Gaucher cells. Typical Gaucher cells also have been found in the spleen and bone marrow of a patient with thalassemia major.<sup>420</sup> Of major importance from the standpoint of pathogenesis was the finding that glucocerebrosidase activity in the white cells and spleen of these patients was normal, thereby eliminating the possibility of associated hereditary Gaucher's disease.<sup>400</sup> Sphingolipid turnover resulting from the daily destruction of erythrocytes and granulocytes in normal man is estimated to be 5 to 10 mg for erythrocytes and 350 to 400 mg for granulocytes.<sup>400</sup> In CML with a granulocyte turnover 5 to 10 times normal, or even greater, the sphingolipid load presented to the monocyte-macrophage system each day must be massive. In some patients this load presumably exceeds the catabolic capacity of the normal reticuloendothelial system, thereby producing Gaucher cells.<sup>379</sup>

### Niemann-Pick Disease

**HISTORY AND PATHOGENESIS.** Niemann-Pick disease, a condition similar to the in-

fantile form of Gaucher's disease, was described in 1914<sup>450</sup> and in 1922.<sup>451</sup> Originally thought to occur only in infants, it has since been found that manifestations of this disease may first appear as late as the second year of life and even later (at six years).<sup>440</sup> A few cases of an adult form also have been described.<sup>418 438</sup> There is a marked predilection for inbred populations; 30% of one series of 18 patients<sup>410</sup> and 50% of another series<sup>461</sup> were of Jewish ancestry. Other patient groups have been identified in non-Jewish families in Canada,<sup>459</sup> Switzerland, and elsewhere.

The excess lipid that accumulates in these patients was shown in 1934 to be sphingomyelin.<sup>417</sup> More recently, Brady and co-workers<sup>437</sup> provided evidence of a deficiency of sphingomyelinase and at least two enzymatically distinct forms of Niemann-Pick disease are recognizable.<sup>437,456</sup> From family studies and tissue enzyme assays these disorders appear to be transmitted as autosomal recessive traits.

**SYMPTOMATOLOGY AND PATHOLOGY.**<sup>350</sup> The clinical picture in Niemann-Pick disease probably includes five different but related conditions.<sup>345</sup> All are characterized by infiltration of the tissues with the characteristic foamy storage cells. These cells are round, oval or polyhedral, and 20 to 90  $\mu m$  in diameter. Their cytoplasm is filled with clusters of small, round droplets (Fig. 42-9 and Plate II, H, p. 72), in contrast to the "wrinkled" cytoplasm of the Gaucher cell. The nucleus of the Niemann-Pick cell is small, eccentrically placed, and there seldom is more than one nucleus in a cell. The vacuoles have a faint bluish hue when treated with Wright's stain; staining with PAS and fat stains gives variable reactions.<sup>452</sup>

Under the electron microscope, small lipid bodies (1 to 2  $\mu m$  in diameter) are seen in histiocytes in the lymph nodes and spleen, and membrane-bound inclusions are seen in hepatocytes, Kupffer cells, and in some cells in the central nervous system. The lamellar structure of the inclusions is less striking than in Gaucher cells.<sup>370</sup>

The involved organs show an increase in

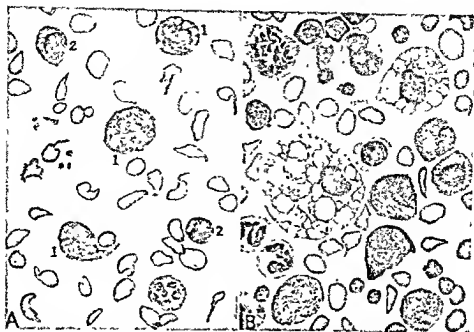


Fig. 42-9 Drawings of representative cells in smears made from (A) peripheral blood and (B) sternal bone marrow in a patient with Niemann Pick disease showing numerous vacuoles in the cytoplasm of monocytes (A, 1) and lymphocytes (A 2) in the blood, and foam cells in the marrow (From Kato<sup>444</sup> courtesy of the author and the American Journal of Diseases of Children)

total lipids, especially sphingomyelin (Fig. 42-5D), but also in other phospholipids and cholesterol.<sup>350,435</sup> The chemical changes in the brain are not uniform or specific.<sup>439</sup>

The classical, infantile or A form of Niemann-Pick disease begins in infancy with signs of mental retardation, hepatosplenomegaly, and sometimes lymphadenopathy; and in about half the patients a cherry-red spot can be seen in the macular region of the fundus oculi.<sup>315,440</sup> Most cases of the disease (perhaps 85%) are of the infantile type.<sup>345,452</sup> About half of those affected are persons of Ashkenazi Jewish origin whose ancestors came from the same Baltic region as did the patients with adult Gaucher's or Tay-Sachs disease. Persistent early jaundice, enlarging abdomen, and poor general nutrition are common initial complaints. Enlargement of the liver and lymph nodes occurs early, in contrast to Gaucher's disease. Difficulty in feeding, bronchitis, and bronchopneumonia mark the course; cachexia develops and death usually occurs by the third year of life.

A characteristic ballooning of neurons in both gray and white matter occurs in the central nervous system.<sup>443</sup> By electron microscopy, these neurons were shown to be filled with numerous membranous cytoplasmic bodies containing large amounts of sphingomyelin. The sphingomyelin content of the spleen and liver also was markedly increased.<sup>445</sup> A severe deficiency of sphingomyelinase has been demonstrated in the liver (0 to 7% of normal), in circulating leukocytes (0%), and in cultured skin fibroblasts,<sup>344</sup> as well as in other tissues.<sup>456</sup>

A more chronic, visceral (B) form is characterized by the onset of hepatosplenomegaly in infancy, but prominent nervous system signs do not develop. Thirteen cases of this type have been described.<sup>345</sup> Sphingomyelin also accumulates in the tissues of these patients and they too are deficient in sphingomyelinase.<sup>345,452</sup>

In a third type, onset is delayed until the subject reaches the age of two to four years, but ultimately visceral and nervous system

changes develop and death occurs during childhood or adolescence.<sup>315,452</sup> Neuronal ballooning is as striking as in group A, but, while sphingomyelin and cholesterol are deposited in the viscera, the concentration of phospholipid in gray matter is normal. Sphingomyelinase activity has not been found to be decreased in these patients.

A fourth type (*the D variant*) was described in patients with a common origin in Nova Scotia.<sup>453</sup> Neurologic signs developed in childhood and progression was slow, with death occurring in adolescence. The enzymatic defect has not been established. Foam cells have been found in heterozygous carriers.

A fifth type, designated E, has occurred in a few adults in whom the disease has a mild chronic course with no neurologic manifestations. In these patients also there is marked accumulation of sphingomyelin and cholesterol. The enzymatic defect is unknown.<sup>345</sup>

As with Gaucher's disease it is possible that defects in different isozymes and differing levels of enzymatic activity explain the varied clinical picture.

**THE BLOOD.** Anemia is not conspicuous and when present is only moderate in degree. Leukopenia may be found, and in some patients leukocytosis was present in the absence of infection.<sup>454</sup> Vacuoles in the cytoplasm of lymphocytes and monocytes may be seen even with the light microscope. They are discrete, round, 0.5 to 1.0  $\mu\text{m}$  in diameter, and often are present in groups of 2 to 15 or even 20 per cell (Fig. 42-9). Rarely, they may distend the cell membrane or displace the nucleus. By electron microscopy they appear to be small lipid-containing cytosomes,<sup>459</sup> the origin of which is not known. Moderate thrombocytopenia also may occur.

Serum lipids usually are normal, but, in some of the patients, cholesterol and phospholipids may be increased.<sup>440</sup>

**DIAGNOSIS.** The clinical picture plus the demonstration of the characteristic foamy cells filled with lipid (Fig. 42-9), which are found in the bone marrow or spleen, are usually sufficient for diagnosis. Measurement

of sphingomyelinase activity in leukocytes, cultured skin fibroblasts, or other tissues<sup>341,347</sup> has proved useful in prenatal diagnosis<sup>347</sup> and in detecting heterozygotes.<sup>347</sup>

**PROGNOSIS AND TREATMENT.** In general, Niemann-Pick disease runs a much more rapid course than does Gaucher's disease. Most patients die within a few months, after a period of severe general deterioration. However, a few have lived to 20 years of age.<sup>410,443</sup>

Treatment is necessarily symptomatic and supportive. Infections are treated as they arise. Splenectomy has been of little value except to relieve symptoms caused by the massive size of the spleen.

### Sea-Blue Histiocytosis

**HISTORY AND PATHOGENESIS.** The term "sea-blue histiocyte syndrome" was coined in 1970 to describe a clinical picture consisting of splenomegaly, mild purpura secondary to thrombocytopenia, and, occasionally, hepatic cirrhosis associated with the presence of numerous histiocytes in the spleen and bone marrow which stain a sea-blue color (Plate II, F, p. 72).<sup>491</sup> The first patient to be reported was a 27 year old man with splenomegaly, numerous blue-pigmented macrophages in the marrow, and a white ring in the macular area of the optic fundi.<sup>490</sup> He has survived with no progression of his disease. Since the next report, in 1954,<sup>495</sup> more than 20 cases have been described.<sup>470,471,476,485,487,490,493</sup> Reports of single cases have predominated, but the disorder has been seen in siblings<sup>471,487</sup> and sea-blue histiocytes were found in four members of one family—the father, mother, and two children.<sup>487,490</sup> Consanguinity was prominent in this family. The disease appears to be panethnic since patients have been reported from America, Europe, Puerto Rico, and Iran<sup>482</sup> and cases have been described in individuals of Caucasian and Negro as well as of mixed ancestry.<sup>487</sup> The frequent occurrence in Puerto Ricans is unexplained. Inheritance is thought to be autosomal recessive.<sup>487</sup>

Tissue lipid analyses in a few patients have shown an increase in phospholipid, sphingomyelin, and total lipid in the spleen and bone marrow.<sup>487</sup> Histochemical studies have shown the presence of lipids containing cerebroside and carbohydrate within histiocytes.<sup>470,478,487,490</sup> In a few patients, increased urinary excretion of mucopolysaccharide was noted, but in others this has not been found.<sup>487</sup> No enzymatic defect has been suggested as a probable cause in this disorder, but low sphingomyelinase activity in the spleen has been reported in one or two patients. The source of the lipid in the storage cells is unknown.

**CLINICAL PICTURE AND COURSE.** Sea-blue histiocytosis may be manifested as a *primary familial syndrome*; or lipid-containing, sea-blue histiocytes may be found in patients with idiopathic thrombocytopenic purpura, chronic myelocytic leukemia, hyperlipoproteinemia, or other associated illnesses, so-called *acquired or secondary sea-blue histiocytosis* (see below):

Considerable variation in the clinical presentation of the *primary* form has been noted. Patients with this disorder usually seek medical attention because of hepatosplenomegaly. The age of detection has ranged from infancy to old age, but most patients have been younger than 40 years old when the diagnosis was made.<sup>490</sup> Macular abnormalities, skin pigmentation, or neurologic manifestations have been reported in only a few. Lung infiltrates suggesting tuberculosis or sarcoidosis have been described in about a third of the patients. Lymphadenopathy is unusual. Purpura is common. Thrombocytopenia is a nearly universal finding. Splenectomy has been carried out to alleviate this condition without apparent exacerbation of the general course.

In most patients the course has been benign and uncomplicated, but in a few there has been progression of disease to involve the bones, lungs, or liver. Death due to hepatic failure or pulmonary disease has been reported and massive gastrointestinal bleeding has occurred.<sup>487</sup>

In general, the younger the age at diagnosis the poorer the prognosis.

**DIAGNOSIS AND PATHOLOGY.** All patients with the primary disorder have numerous sea-blue histiocytes diffusely infiltrating the bone marrow. In most of them, these cells have been found in the spleen and the liver. Diffuse and widespread infiltration of the splenic white pulp is usually noted, but may be absent in older patients.<sup>487</sup> No consistent pattern is found in the liver or lymph nodes. Lipid histiocytes have also been found in the lungs in about one third of the patients.

The typical sea-blue histiocyte (Plate II, F) is a large reticuloendothelial cell of 20 to 60  $\mu\text{m}$  diameter with a single eccentric nucleus containing block chromatin and a single nucleolus. When stained with Wright's or Giemsa stain the cytoplasm is seen to contain sea-blue or blue-green granules in varying numbers. Histochemically the granules stain with Sudan black B, other lipid stains, PAS, and acid-fast stains<sup>478,487</sup> and exhibit autofluorescence.<sup>466,478</sup> Electron microscopy reveals lamellae of lipid molecules with a characteristic periodicity.<sup>478,487</sup>

**ACQUIRED OR SECONDARY SEA-BLUE HISTIOCYTOSIS.** Although the familial occurrence and the clinical picture just described suggest the existence of a syndrome secondary to an as yet undefined metabolic defect, sea-blue histiocytes are not specific for this disorder.<sup>480,486</sup>

In a few patients with idiopathic thrombocytopenic purpura, similar if not identical cells have been found in the spleen,<sup>463,474,484</sup> but their presence in the bone marrow has been noted in only one patient.<sup>480</sup> In one study, lipid histiocytes were found in six of 737 spleens removed for various causes.<sup>484</sup> All six patients had thrombocytopenic purpura. Curiously the characteristic cells were not found in the spleens of patients splenectomized before 1953. This led to the suggestion that the sea-blue histiocytes might be related to the therapeutic use of adrenal corticosteroids, but in patients with the disease who were not so treated such cells also have been reported.<sup>484</sup> No association with the

duration of the thrombocytopenia, age of the patient, or other clinical parameters could be established.<sup>466,481</sup> In another study, when autofluorescence was used as a means of detecting lipid-laden histiocytes, these cells could be demonstrated in more than half the patients splenectomized because of ITP.<sup>466</sup> An arrangement of histiocytes around the periphery of the malpighian bodies is said to be a characteristic in patients with ITP.<sup>466</sup> Electron microscopy revealed numerous myelin figures and platelets in various stages of degeneration in the histiocytes found in spleens removed from eight of nine patients with ITP.<sup>468</sup>

Sea-blue histiocytes have been identified also in the spleens of occasional patients with thalassemia (Chapter 26, page 859), chronic myelocytic leukemia,<sup>378</sup> polycythemia vera, sickle cell anemia, sarcoidosis, chronic granulomatous disease,<sup>473</sup> lipochrome histiocytosis, hyperlipoproteinemia, Wolman's disease, hereditary acyltransferase deficiency, or various lipidoses.<sup>480,483,490</sup> We have seen them in a patient with multiple myeloma.

It is not yet clear whether secondary sea-blue histiocytosis is the result of overloading of normal enzyme systems or is due to a mild enzymatic deficiency.

### Fabry's Disease (Angiokeratoma Corporis Diffusum Universale)

**HISTORY AND DEFINITION.** This disorder was first described by Fabry<sup>516</sup> and by Anderson<sup>500</sup> in 1898 as a peculiar nodular, purpura-like skin lesion. Over the next 25 years, several more cases were reported.<sup>517</sup> Ultimately the condition emerged as a clinical syndrome characterized by skin lesions, usually distributed over the scrotum, ilio-sacral region, thighs, and around the umbilicus, occurring in males only, which is also usually associated with albuminuria and edema of the lower extremities or around the eyes. Pain, cramps, numbness, and flushing of the extremities in warm weather and cardiac enlargement also were noted in the affected men. Microscopic sections of the skin lesions revealed dilated vascular channels in the epi-

thelium often with a hypertrophic stratum corneum overlying them. A familial occurrence was noted and thickening of the media of medium-sized and large arteries, especially prominent in the kidneys, with vacuolization of smooth muscle fibers in vessel walls and the myocardium and accumulation of hyaline material therein was described.<sup>534,536</sup> Thus the systemic nature of the disorder was established and a metabolic defect, perhaps a thesaurismosis, was postulated.<sup>534</sup> The nature of the stored material was not identified until the early 1950's when the phospholipid nature of the birefringent deposits was recognized.<sup>517</sup> Fessas and associates, in 1955, were the first to report the disorder in an American patient, who was referred to them because of "purpura" (Fig. 42-10A), anemia, and albuminuria. Intracellular birefringent bodies were demonstrated in this patient's urine and "foam" cells were found in his bone marrow (Fig. 42-10C, D).<sup>517</sup> Studies of an English family demonstrated the typical renal lesion in females as well as males,<sup>512</sup> a finding subsequently confirmed.<sup>533</sup> By 1961 about 35 cases had been reported and the pattern of inheritance was shown to be sex-linked recessive.<sup>529,532,535</sup> In 1967 the enzymatic defect was established as ceramide trihexosidase or  $\alpha$ -galactosidase deficiency (Fig. 42-6A).<sup>507</sup> Cultures of skin fibroblasts from a patient's mother and sister demonstrated two distinct clonal populations, one with and one without enzymatic activity.<sup>537</sup> This provided evidence of gene inactivation (Lyonization) in female carriers, whose intermediate levels of 15 to 40% of normal enzymatic activity<sup>521</sup> probably explain the usually mild nature of the disease in the female. It is not yet clear whether the X-linked defect is in a structural or regulator gene.<sup>505</sup> The fact that plasma infusions from normal subjects lead in several hours to an increase in plasma ceramide trihexosidase activity to over 150% of normal in patients with Fabry's disease suggests that the defect may be in a regulator gene.<sup>505,523,528</sup>

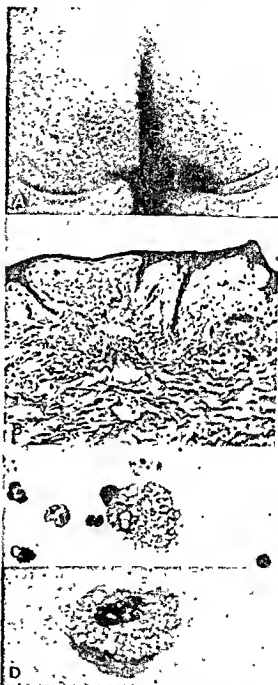
**ETIOLOGY, PATHOLOGY, AND PATHOGENESIS.** The deficiency in  $\alpha$ -galactosidase<sup>505,507</sup>



leads to accumulation of ceramide trihexose in many cells, especially smooth muscle cells in the walls of the blood vessels, in cardiac muscle, and in renal epithelium, as well as in bronchial epithelium, neurons, histiocytes, and endothelium throughout the body.<sup>501,512,519-534</sup> These deposits can be seen following PAS or sudan black B staining of vessel endothelial cells, histiocytes, or smooth muscle cells.<sup>519</sup> Under the electron microscope, myelin figures with a periodic structure similar to that found in some of the other lipidoses are seen.<sup>519</sup> However, the source of the cerebroside is not at all clear and the tissue distribution is quite different from that seen in the other lipidoses. The absence of associated amino acids and plasma proteins in vessel walls has been thought to rule out the possibility of plasma lipoprotein as a source.<sup>525</sup> An apparent increase in pinocytotic activity suggests that glycolipid is taken up from surrounding tissue fluids. The increased blood and urine ceramide trihexose levels ( $10 \times$  normal) are compatible with this thesis. However, the lack of membranes surrounding many of the inclusions in endothelial cells and fibrocytes is not compatible, unless one postulates secondary rupture of phagolysosomes with liberation of incompletely degraded contents into the cell cytoplasm.<sup>530</sup> Since globosides are normally present in blood cells and kidney parenchyma while gangliosides are major constituents of neural tissue, and since there are no lipid deposits in neural cells, it has been suggested that Fabry's disease results from a block in globoside degradation.<sup>526</sup> According to this concept the CNS changes are secondary to deposits and changes in the blood vessels.

**CLINICAL PICTURE AND COURSE.**<sup>539</sup> The main features of the clinical picture were mentioned above. The course can be divided into three phases.<sup>535</sup>

The *childhood or adolescent phase* is characterized by crises of fever, burning discomfort of the hands and feet, paresthesias, proteinuria, and the appearance of the typical vascular lesions in the conjunctival vessels,



**Fig 42.10** A, Typical skin lesions on the buttocks of a patient with Fabry's disease. B, Subepidermal vascular aneurysm from skin of the patient shown in A. (Magnification approximately  $\times 200$ ) C, Vacuolated macrophage in bone marrow aspirate. D, Vacuolated cell in urinary sediment. (From Fessas, Wintrobe, and Cartwright,<sup>517</sup> courtesy of the authors and the American Medical Association.)

lips, and skin. These manifestations occur only in males. The crises of fever are commonly associated with limb pains and are precipitated by hot or cold weather. The pain is mainly in the skin of the fingers and toes and does not usually spread proximally. Episodes may be brief or last for several weeks. In some subjects the pain has been of such severity that suicide has been attempted because of it.

The skin lesions are not usually a source of complaint, but they are of paramount importance in diagnosis. They are punctate, blood-filled angiectasias and form in the superficial layers of the skin (Fig. 42-10A, B). They are dark red to almost black in color and their surface is shiny. Their blood content can be only partially expressed by compression, and minimal bleeding follows trauma or pricking with a pin. These lesions characteristically occur in clusters especially over the scrotum, around the umbilicus, and over the thighs and buttocks. As the disease progresses they become more numerous and more extensive in distribution. The disease (or variants thereof) may occur without skin lesions<sup>509</sup>; this is the usual pattern in females, but may be encountered in males as well. The venules in the conjunctivae and the fundus oculi show tortuosity, dilatation, and sacculatation and corneal opacities are seen on careful slit-lamp examination.<sup>535</sup> Some edema is present in most affected males, and proteinuria is common. Vacuolated macrophages may be seen in the urine (Fig. 42-10D).<sup>517</sup> Anhidrosis may be noted in some patients and presumably explains the discomfort experienced by them in hot weather or after exertion.

During the *quiescent phase*, which is observed in early adult life, the crises cease, but proteinuria, cylindruria, and deterioration of renal function continue and anhidrosis becomes more prominent. The skin lesions increase in number.

The accelerated phase develops in adult life and is characterized by progressive renal failure, hypertension, cardiomegaly, and finally death in the fourth or fifth decade of life,

usually from renal failure or vascular accidents.

Cerebral symptoms and even cerebral vascular occlusions or myocardial infarctions may occur in a young patient, and varicose veins and hemorrhoids are common. Although pulmonary symptoms have been attributed to Fabry's disease there is little evidence to support this claim.<sup>503</sup>

In the heterozygous females, clinical manifestations are lacking.

**LABORATORY FINDINGS.** A moderate degree of anemia is common. It is usually hypochromic and possibly results from the bleeding hemorrhoids, but it may be normochromic and normocytic.<sup>519</sup> The findings in the bone marrow and urine have been mentioned already.

**DIAGNOSIS.** The clinical picture is so characteristic that in male subjects the diagnosis of Fabry's disease can usually be made if this disease is thought of. The skin lesions, however, have been mistaken for purpura. The diagnosis can readily be confirmed by chemical analysis of plasma<sup>547</sup> or of urinary sediment for the abnormal sphingolipid. Heterozygous carriers also can be detected by these means.<sup>514</sup> Tissue cultures can be assessed for accumulation of the poorly catabolized lipid, or the level of the enzyme ceramide trihexosidase can be measured.<sup>547,507</sup> Specific enzyme assay should probably be used since variants occur.<sup>509</sup>

**TREATMENT.** No treatment has been devised to prevent the development and progression of Fabry's disease. Infusions of normal plasma have produced transient increases in serum ceramide trihexosidase activity and decreases in serum ceramide trihexose levels,<sup>523,529</sup> but no long-term improvement has resulted. A few uremic patients have received renal allotransplants with some benefit, but whether or not the transplanted kidney can correct the basic metabolic defect is moot.<sup>510,533</sup>

As with many of the other lipoidoses, amniotic fluid cell analysis permits identification of an affected, hemizygous fetus.<sup>547</sup>

## Histiocytosis X

### Introduction and History<sup>592,619</sup>

The clinical triad of defects in membranous bone, exophthalmos, and polyuria in children, subsequently known as *Hand-Schüller-Christian disease*, was outlined in 1921 in a review by Hand.<sup>575</sup> In this review, Hand described the first six cases, including those of Christian and of Schuller and his own first case reported in 1893.<sup>574</sup> All six patients had hepatosplenomegaly, lymphadenopathy, and bone lesions, but not all had exophthalmos and polyuria. Attempts to link the disorder to xanthoma tuberosum, lipid storage disease,<sup>605</sup> and the xanthomatoses<sup>616</sup> proved unsuccessful and to this day there is no evidence that a specific biochemical defect is responsible for the condition. However, the "foamy" or "xanthoma" cell came to be regarded as a pathognomonic feature of the syndrome.<sup>592</sup>

A rather different clinical entity, consisting of fever, bilateral otitis media, hepatosplenomegaly, and adenopathy in a young infant who soon died was described by Letterer in 1924.<sup>588</sup> In 1933, Siwe described a condition in a 16 month old girl who died after a three-month illness during which she had fever, hepatosplenomegaly, lymphadenopathy, neutrophilia, and a destructive bone lesion in a fibula.<sup>611</sup> At autopsy, massive infiltrates of large cells resembling histiocytes were found. Siwe reviewed five other cases from the literature (including that of Letterer) and concluded that they constituted a single clinical entity.

In 1940, two groups of investigators<sup>591,602</sup> called attention to a syndrome in which there was infiltration of bone by eosinophilic granulomas. Several years later, Farber and co-workers<sup>564,573</sup> described a group of patients with solitary *eosinophilic granuloma of bone* the lesions of which usually healed promptly after irradiation or curettage. These authors concluded that their cases fitted a spectrum of disease, including Letterer-Siwe and the Hand-Schüller-Christian syndrome. In 1953,

because of the similarity of the histiocytosis observed in these three disorders, Lichtenstein<sup>577,590</sup> grouped them under a single heading which he designated "histiocytosis X" to indicate their unknown cause. At first well received,<sup>546,584,595</sup> this unifying concept has been contested by some workers<sup>601,612</sup> and has lost favor.<sup>592,612</sup> The cause of these conditions is still unknown.

### Eosinophilic Granuloma

Unifocal or multifocal granulomas, which on pathologic section exhibit a mixture of histiocytes and mature eosinophils, are the distinguishing features of eosinophilic granuloma, the most common of the three entities under consideration.<sup>547,592</sup> In one study of 82 patients with the three syndromes, 74 were noted to have eosinophilic granuloma. Of these, 50 had unifocal and 24 multifocal granulomas.<sup>592</sup>

#### Unifocal Eosinophilic Granuloma of Bone

These lesions have been observed in patients of all ages (16 months to 61 years). Of the 50 patients mentioned above, 27 were children under the age of 10 years and in all but six the disease occurred before the subjects were 40 years old.<sup>592</sup> Males were affected more commonly than females (ratio 3:2). Lesions were found in many different bones, but the head, ribs, and femurs were the most common sites (Table 42-3). The ribs were most frequently affected in adults, while the head and femurs were the usual locations in children.

The clinical picture and course of the disease are characterized by pain, tenderness and sometimes swelling over the affected bony site. X-ray examination reveals rarefaction in the medullary areas of the membranous or long bones. As the lesion enlarges it erodes the inner table of the cortex and resembles a cyst (Fig. 42-11). In some of the patients, reactive sclerosis may develop. Pathologic fracture and vertebral collapse may occur. Occasional cases may mimic sarcoma of

**Table 42-3. Sites of Lesions in Unifocal Eosinophilic Granuloma of Bone<sup>592</sup>**

Site	< 15 Years	> 15 Years	Total
Head	7	3	10
Scapula	1	1	2
Clavicle	1	1	2
Humerus	2	1	3
Radius	1	—	1
Spine	2	—	2
Ribs	1	9	10
Ilium	5	—	5
Pubis	2	—	2
Femur	9	2	11
Tibia	2	—	2

bone.<sup>519</sup> Generally there are few if any constitutional symptoms and in a series of 50 patients none exhibited exophthalmos or symptoms of diabetes insipidus.<sup>592</sup>

There are no helpful laboratory findings and there is no associated blood eosinophilia. *Diagnosis* is made by means of biopsy. Simultaneous curettage often suffices as treatment for these solitary lesions.<sup>599</sup> Modest doses of x-ray therapy (500 to 1500 rads) also have been reported to be helpful, while some lesions heal without therapy.<sup>599</sup> Radiotherapy is the *treatment* of choice when the lesions involve vital areas such as the neck of the femur, ramus of the mandible, or vertebral bodies and areas such as orbital lesions where good cosmetic results are important. In such cases, low dosage (300 to 600 rads) is recommended.<sup>599</sup>

#### *Unifocal Eosinophilic Granuloma in Sites Other Than Bone*

Solitary lesions characterized by granuloma formation with marked eosinophilic infiltration have been reported in tissues other than bone, especially the lungs and the gastrointestinal tract. Extraordinarily rare sites of involvement are the thymus, urinary bladder, parotid gland,<sup>550</sup> hypothalamus,<sup>551</sup> and skin.<sup>597</sup> In the gastrointestinal tract, solitary lesions may simulate polyps, gastric carcinoma, or gastric or duodenal ulcer.<sup>607</sup>

*Unifocal pulmonary eosinophilic granuloma* appears predominantly in young white males.<sup>553,617,622,624</sup> The onset is usually sudden and symptoms consist of cough, weight loss, and dyspnea. Some patients are asymptomatic and their disease then is detected only by x-ray examination, which reveals discrete or coalescent nodular infiltrates and small cystic areas. Lung biopsy is required for diagnosis. Electron microscopy has revealed peculiar rods in some lesions.<sup>571</sup> The acute phase of the disease usually subsides spontaneously after a few months, leaving residual interstitial fibrosis. Spontaneous pneumothorax occurs in 20 to 50% of the patients.<sup>622</sup>



**Fig 42-11.** Radiolucent eosinophilic granuloma of humeral diaphysis with periosteal reaction. Ewing's tumor was a differential diagnostic problem. (From Ochsenr,<sup>599</sup> courtesy of the author and Charles C Thomas.)

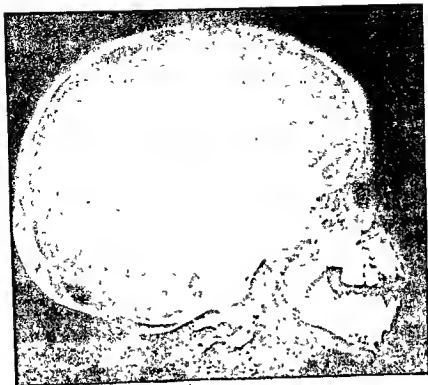


Fig 42-12. Osteolytic lesions in the skull of a patient (D E J) with Letterer-Siwe disease

Adrenocorticosteroid or x-ray therapy has been reported to be of some therapeutic benefit.<sup>553</sup>

#### *Multifocal Eosinophilic Granulomas of Bone and Schüller-Christian Disease*

Patients of all ages (1 month to 57 years) may be affected with multiple eosinophilic granulomas, but most, 22 out of 24 in one series<sup>592</sup> and 18 of 29 in another,<sup>547</sup> developed symptoms before the age of five years. Males predominate to some degree.<sup>547,592</sup>

If a patient with unifocal disease is going to develop additional lesions this usually occurs within six months of the finding of the first lesion.<sup>592</sup> The classic clinical picture of Hand-Schüller-Christian disease, namely, exophthalmos, diabetes insipidus, and bone lesions, was seen in only six of 24 patients with multifocal disease,<sup>592</sup> but symptoms either of diabetes insipidus or of exophthalmos were somewhat more common, being seen in one third to one half of the patients.<sup>547,592</sup> When

carefully sought, pituitary dysfunction is found in most patients.<sup>556</sup> Lymphadenopathy, splenomegaly, or hepatomegaly is present in 25 to 50% of the patients, as is dermatitis. Most develop fever during the acute phases of the illness. The disease is chronic in course, however, and usually begins with a *chronic otitis media*. Other modes of presentation include polydipsia and polyuria (diabetes insipidus), together with increasing irritability, exophthalmos, extrusion of teeth in association with bone lesions in the jaw, and even dermatitis.

*Defects in membranous bones* are manifested chiefly by sharply defined lesions in the skull, which often assume a geographic pattern (Fig. 42-12). The face may be deformed by involvement of the bones of the orbit. Erosions of the mandible are relatively common, usually the tooth-bearing portions, and ultimately displacement of the teeth by the tumor causes them to appear in roentgenograms as if suspended in space. Involvement of the mastoid bone in association with the

chronic otitis media is also very common. Other bones that may be involved include the pelvis, femurs,<sup>592-598</sup> ribs, humerus, and spine.<sup>547</sup> In the *long bones* the lesions occur toward the ends of the shafts. There is thinning from within and, in contrast to the lesions in membranous bone, periosteal reaction often occurs.<sup>547</sup> *Exophthalmos* develops as a consequence of orbital tumors. Symptoms of *diabetes insipidus* are presumably due to involvement of the pituitary gland or hypothalamus; associated destruction of the sella turcica is infrequent.

*Skin and mucous membrane* lesions include ulcerations that resemble those seen in the Letterer-Siwe syndrome<sup>625</sup> or xanthoma disseminatum. Those resembling xanthoma disseminatum consist of painless, yellowish-brown, maculopapular lesions that may be scattered over the face, especially the eyes and mouth, trunk, perineum, and axillae. Fibrotic lesions associated with extracellular lipid deposits (presumably late-stage healing lesions) may be found in the mouth, under the tongue, and in the pharynx and larynx.<sup>581</sup>

Involvement of the *lungs* may be perihilar, central, or diffuse. Bilateral interstitial pulmonary infiltration leads eventually to fibrosis,<sup>603</sup> honey-combing of the lungs, and episodes of spontaneous pneumothorax; the course is variable,<sup>589</sup> but alveolocapillary block, cor pulmonale, and right heart failure may develop. Pleural involvement is unusual.

Moderate enlargement of the *liver, spleen, and lymph nodes* may occur. Gastrointestinal lesions can simulate ulceration of the stomach or duodenum, or polyps or carcinoma.<sup>607-613</sup> Actual involvement of the nervous system is rare<sup>547-561</sup> except for the hypothalamus.

Anemia may or may not be found. When it occurs it is of the non-regenerative type<sup>578</sup> and may be accompanied by leukopenia or thrombocytopenia. Rarely myelophthisic anemia may be noted. In one subject,  $\gamma$ -paraproteinemia was observed and led to a mistaken diagnosis of multiple myeloma.<sup>582</sup>

*Diagnosis* may be difficult to establish unless a characteristic combination of signs is present. Outstanding features in various

combinations and with or without signs of wasting disease are: skeletal lesions; cutaneous, oral, or anogenital manifestations; hepatosplenomegaly; adenopathy; exophthalmos; and diabetes insipidus. A bone lesion may develop so rapidly and break through the cortex so readily that prior to biopsy it may be mistaken for a malignant tumor, such as Ewing's sarcoma. Multiple foci may suggest metastatic neuroblastoma. Chronic otitis media, intractable seborrhea, or milium pulmonary infiltrates also may lead one astray. The diagnosis ultimately rests on histologic examination of involved tissue.

*Pathologically*, lesions such as those in the skull are found to consist of defects, of various shapes and size, which are filled with a light yellow or brownish, tough substance. Similar granulation tissue is found in the spleen, liver, lymph nodes, and other tissues. The basic microscopic picture of the bone lesions, whether uni- or multifocal, is that of a mixture of mature eosinophilic leukocytes and histiocytes in varying proportions.<sup>592</sup> The histiocytes may or may not contain considerable phagocytized cellular debris. In many specimens the mononuclear cells vary in size, contain abundant cytoplasm, and may take on a syncytial appearance. Mitotic figures are unusual; occasional giant, multinucleated histiocytes are seen. It is uncommon to find more than an occasional foam cell or xanthoma cell in the bone lesions. Such cells are present primarily when there has been *chronic suppuration (as in chronic mastoiditis)* in which case they are found interspersed among fibroblasts.<sup>592</sup> Ultimately there is a decrease in the number of eosinophils and a tendency to healing and fibrosis. If one searches carefully, Charcot-Leyden crystals may be demonstrable.<sup>549</sup>

The skin lesions of eosinophilic granuloma may have the characteristic appearance of eosinophilic granuloma, or an almost purely histiocytic reaction may be noted. In the latter case it is difficult to make the diagnosis and confusion readily occurs with the lesions seen in the more malignant Letterer-Siwe disease.<sup>592</sup>

The ultimate prognosis is better<sup>547,592</sup> than once was thought.<sup>613</sup> The outlook may be less good when the condition begins early in life<sup>584</sup> and death may result from pulmonary fibrosis, pneumothorax, or other causes. However, spontaneous modest improvement or even remissions have been observed. Thus in one series of 29 cases, one third of the patients recovered, one half continued to have active disease, and 13% died.<sup>547</sup> In a more recent series of 24 cases<sup>592</sup> only one patient died, of an unrelated cause. In general the prognosis can be related to the extent of the disease process, the number of tissues involved<sup>584</sup> and whether or not there is impairment of organ function.<sup>586</sup>

As these syndromes have been studied more carefully, it has become apparent that a child with a solitary "eosinophilic granuloma of bone" may develop signs suggesting systemic disease, and similar lesions may be found in extra-skeletal sites such as lymph nodes, skin, oral cavity, lungs, or the anogenital region. However, the transition from solitary to multifocal lesions appears to occur in less than half of the cases.<sup>592</sup>

**TREATMENT.** As already mentioned, spontaneous healing of lesions may occur and simple curettage or small doses of x-ray therapy given to localized lesions<sup>599</sup> may induce a favorable response. Systemic therapy with methotrexate, vinca alkaloids, corticotropin,<sup>565</sup> adrenal steroids,<sup>548</sup> cyclophosphamide, or nitrogen mustard is usually advised to control the widespread lesions.<sup>585,592</sup> Adrenal corticosteroid therapy is capable of reversing all the skeletal and visceral manifestations when large doses (2 to 4 mg of prednisone per kg of body weight per day) are administered for six- to eight-week periods. With such therapy, remissions were obtained and sustained for 12 to 30 months.<sup>548</sup> Vinblastine sulfate in doses of 0.2 to 0.3 mg/kg given weekly has also been effective<sup>610</sup> as is vincristine (2 mg/m<sup>2</sup> weekly) or cyclophosphamide given orally (5 mg/kg per day).<sup>614</sup> With these forms of therapy complete remissions, perhaps cures, have

been achieved in 20 to 50% of the patients and partial remission in an additional 50 to 60%.<sup>614</sup>

**PATHOGENESIS.** Most students of this disorder regard it as an inflammatory granuloma, possibly of infectious origin, rather than a metabolic disorder as once assumed. Electron microscopic studies have failed to demonstrate virus particles,<sup>604</sup> but lysosomes, myelin figures, and endoplasmic reticulum are prominent throughout the cytoplasm of the histiocytes.<sup>604</sup> There is no evidence of consanguinity in the families of affected patients.<sup>566</sup>

### *Letterer-Siwe Disease*

The term "Letterer-Siwe disease," as originally used, described an acute illness of unknown cause<sup>545</sup> characterized by: (1) occurrence in infants exclusively, (2) marked splenomegaly, considerable hepatomegaly, lymphadenopathy, (3) localized bone tumors, often with erosion demonstrable by x ray, (4) anemia with normal or decreased platelets, unremarkable leukocyte differential count, a hemorrhagic diathesis, and (5) increased numbers of nonlipid histiocytic cells in splenic aspirates. Subsequent studies have modified these criteria.

Letterer-Siwe disease occurs less frequently than does solitary or multifocal eosinophilic granuloma. It has been observed in young children as well as infants, and occasional cases have been reported in older children.<sup>547,584,592</sup> A few cases have been described in neonates.<sup>560</sup>

**CLINICAL PICTURE.** There is evidence of a wasting disorder with enlargement of the spleen, liver, and lymph nodes. Moderate fever is often present. Exophthalmos and diabetes insipidus are less prominent in this disorder than in Schüller-Christian disease,<sup>592</sup> but all patients have had skull lesions like those described in that condition. *Bone lesions* occur in many other sites as well, the upper extremities being commonly affected.<sup>562</sup> *Skin* involvement is particularly

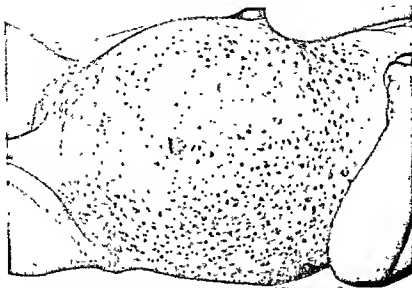


Fig 42-13. The typical rash of Letterer-Siwe disease—papular, scaly, crusted lesions predominantly on the trunk with confluent erythematous lesions in the groin (From Lahey<sup>585</sup> courtesy of the author and Harper & Row)

prominent in Letterer-Siwe disease (Fig. 42-13) and not infrequently is the presenting complaint.<sup>625</sup> The lesions are papular, scaly and crusted and are seen predominantly on the trunk and over the scalp; they may ulcerate or, on occasion, become hemorrhagic. *Lymphadenopathy* appears to result from direct histiocytic infiltration rather than due to secondary involvement from more distal lesions.<sup>564</sup>

**LABORATORY FINDINGS.** Anemia is common in this syndrome, thrombocytopenia is frequent, leukopenia may occur, and pancytopenia has been reported.<sup>594</sup> The anemia is normochromic normocytic and is often hemolytic.<sup>565</sup> The pathogenesis of the thrombocytopenia is not clear, there being no good correlation with the degree of marrow involvement or the number of megakaryocytes in the bone marrow.<sup>562</sup> Monocytosis may occur.<sup>585</sup> Hemohistiocytes have been seen in the blood<sup>600</sup> and a leukemic picture has been observed.<sup>567,585</sup>

**DIAGNOSIS.** Widespread involvement of bone, skin, and the reticuloendothelial system in infants, with histiocytic infiltration demon-

strable on biopsy, establishes the diagnosis. However, when involvement is in atypical sites or is less extensive than usual, confusion concerning the diagnosis may arise. Thus the skin lesions may be mistaken for seborrheic dermatitis; the bone lesions may be confused with neoplasms or osteomyelitis; and the lung infiltrates may be mistaken for those due to tuberculosis, histoplasmosis, or cystic fibrosis. Spleen puncture is useful mainly in ruling out the possibility of lipid storage diseases,<sup>619</sup> but skin biopsy may be misleading.<sup>592</sup>

**PATHOLOGY.** The pathologic picture of Letterer-Siwe disease has not been clearly delineated and there is considerable overlap between the lesions in this condition and those of multifocal eosinophilic granuloma.<sup>592</sup> In the latter, there is a mixture of eosinophils and histiocytes with indistinct cell membranes forming sheets with a polygonal shape, which produce a syncytial appearance.<sup>586</sup> In contrast, in Letterer-Siwe disease there is diffuse infiltration by cells with abundant cytoplasm and distinct cell membranes, together with rare giant cells,



eosinophils, or fibrosis. There is no tendency to develop a syncytial appearance.<sup>586</sup>

**PROGNOSIS, COURSE, AND TREATMENT.** Depending on the type of cases included under this category, whether only acute cases or also the more chronic ones, Letterer-Siwe disease has been regarded as uniformly fatal<sup>545,592</sup> or the prognosis has been considerably better than had been thought in the past.<sup>594</sup> Mortality is low in patients with the least extensive disease and high when the disease is widely disseminated. Onset before the age of six months appears to carry a bad prognosis. The overall mortality in one series of 69 patients was 34%. No patient over three years of age at onset of the disease died during the course of that study.<sup>584</sup>

When the disease is widespread and extensive, treatment must be aggressive. The use of vincristine (2 mg/m<sup>2</sup> weekly) or vinblastine intravenously (6.5 mg/m<sup>2</sup> initially, with subsequent dosage modified according to toxic side effects), or of cyclophosphamide orally (5 mg/kg/day), resulted in complete remission in 20 to 50% of the patients in one series and partial remissions in 50%.<sup>611</sup> However, good results also have been reported in patients treated with antibiotics,<sup>554</sup> prednisone,<sup>548</sup> or a variety of other chemotherapeutic agents.<sup>585</sup>

**PATHOGENESIS.** Because of the rapidly fatal course, this syndrome is regarded by some as a neoplastic process.<sup>592,619</sup> However, the occasional response to antibiotics suggests an underlying infectious process.<sup>554</sup> The concentration of deaths in the first two years of life and the occurrence in siblings and in several sets of twins<sup>570,619</sup> as well as in neonates have been taken to indicate a prenatal origin, perhaps viral infection.<sup>570,619</sup>

Electron microscopy has revealed peculiar atypical granules in the histiocytes in this disorder,<sup>558,608</sup> but similar ones also have been found in normal skin and in other tumors.<sup>619</sup>

Several studies have suggested genetic inheritance.<sup>568,579</sup> The above-mentioned incidence in siblings and twins<sup>570</sup> indicates the

need for careful study of family members and of chromosome constitution.

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## Infectious Mononucleosis

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**I**NFECTIONOUS mononucleosis is an acute sporadic infection that usually affects young adults. It is accompanied by irregular fever, severe pharyngitis, adenopathy, and splenomegaly. Characteristic laboratory findings include an absolute lymphocytosis with many "atypical" cells, antibodies against heterologous red cells, and rising titers against the Epstein-Barr virus.

### History and Terminology

In the first decade of this century, several cases of glandular enlargement, sore throat, and mononuclear cell increase were described by Turk,<sup>280</sup> Cabot, Marchant, and others,<sup>295</sup> sometimes as examples of acute leukemia with apparent cure. It remained for Sprunt and associates<sup>290</sup> to classify these cases and six of their own under the title "*infectious*

*mononucleosis*" and to point out that blast-like cells of a peculiar type were present in the blood. A detailed morphologic description of these cells was later published by Downey and coworkers in 1923.<sup>68</sup>

An important advance was made in 1932 when Paul and associates discovered that the sera of patients with infectious mononucleosis contain antibodies against sheep erythrocytes in concentrations far above normal.<sup>221</sup> Equally important has been the more recent discovery of the association between infectious mononucleosis and the Epstein-Barr virus<sup>70,80,192</sup> (see below).

In many textbooks and articles it is still stated that infectious mononucleosis was first described by Pfeiffer<sup>226</sup> in 1889 as "Drüsenfieber," a "glandular fever" of epidemic nature, and that subsequently the condition was also reported by others.<sup>15,24,293</sup> Tidy and colleagues<sup>276</sup> suggested that glandular fever and infectious mononucleosis are one and the same. However, a careful analysis of clinical data by Hoagland<sup>143</sup> showed pronounced differences between these two conditions. *Glandular fever* is highly contagious, usually affects children, and is of short duration. The throat is only mildly inflamed and adenopathy may be restricted to the cervical nodes only. The spleen is not enlarged, except when the illness is very protracted. Thus, on clinical and epidemiologic grounds alone, infectious mononucleosis is clearly separable from Pfeiffer's Drüsenfieber (see below).



## Etiology

The search for an etiologic agent in infectious mononucleosis has exemplified Sherlock Holmes' dictum that "it is a capital mistake to theorize before one has data." Suspected etiologic agents have ranged from cocci and bacilli to spirochetes, protozoa, and viruses.<sup>295</sup> Most intriguing has been the association between sporadic, heterophil-positive infectious mononucleosis and a herpes-like agent, which was first described in 1964 by Epstein, Achong, and Barr as present in lymphoblasts cultured from patients with Burkitt's lymphoma<sup>79,80</sup> (see also page 1452). These findings were subsequently confirmed by others<sup>129,164</sup> and the agent is now commonly referred to as the Epstein-Barr virus (EBV). Although its association with Burkitt's lymphoma remains most firmly established, it has also been identified in lymphoid cell lines derived from patients with infectious mononucleosis,<sup>192</sup> patients with various lymphomas,<sup>22</sup> those with leukemia,<sup>10</sup> those with sarcoidosis,<sup>133</sup> and from persons presumably in good health.<sup>197</sup> Interestingly, the EB virus is readily observed in lymphoid cell cultures derived from these subjects, but it rarely is seen in fresh biopsy material; nevertheless, the sera of patients with Burkitt's lymphoma,<sup>129,132,170,263</sup> of those with carcinoma of the postnasal space,<sup>66,133,215</sup> and of those with infectious mononucleosis<sup>5,192,205,267</sup> contain large quantities of anti-EBV antibodies suggesting that the presence of the virus in *in vitro* culture systems is not just an artifact. However, a firm etiologic relationship between such conditions and the EBV has not yet been established.<sup>10,22,133</sup> Indeed, low titers of anti-EBV antibodies may be seen in well over half of all normal individuals in a worldwide distribution.<sup>129,132,181,192,206,223</sup>

The implication of the EB virus as the cause of infectious mononucleosis is the result of a chance observation made in 1967<sup>131</sup>. Blood taken from a laboratory technician who had contracted infectious mononucleosis led to the establishment of a lymphoid cell line containing the characteristic EB virus. Sig-

nificantly, previous attempts to establish a lymphoid cell line from this technician's blood had been unsuccessful and sera obtained prior to the onset of clinical infectious mononucleosis had shown anti-EBV titers of less than 1:10 and a negative heterophil response. Following the onset of infectious mononucleosis, anti-EBV and heterophil titers rose. On the basis of these provocative clues, Henle and associates studied sera of a number of students from Yale University, where a prospective study of infectious mononucleosis had been in progress for many years. In all instances of clinical infectious mononucleosis the anti-EBV and heterophil titers rose together from a negative baseline. Of even greater interest was the observation that the anti-EBV titer remained persistently elevated in contrast to the transient nature of the heterophil response.

Since then other reports have provided further sero-epidemiologic evidence for the role of EB virus in infectious mononucleosis.<sup>5,83,105,139,178,205,246,255</sup> This evidence may be summarized as follows: (1) Typical heterophil-positive infectious mononucleosis is almost always associated with a rise in the titer of antibodies to EBV-related antigens.<sup>5,125,136,205</sup> In heterophil-negative "mononucleosis" the response is variable, but more commonly negative.<sup>5,83,169</sup> Other viral infections are not characterized by anti-EBV antibodies.<sup>83,130</sup> Antibodies to EBV-related antigens include those against EB-viral capsid antigens (VCA)<sup>126,129</sup>; antibodies to EBV-determined cell membrane antigens<sup>164</sup> which are comparable to some tumor specific antigens (TSA) of virus-induced tumors (page 329); antibodies to the D component of the EBV-induced early antigen (EA) complex<sup>132a</sup>; complement-fixing antibodies to soluble and viral antigens<sup>103</sup>; and EBV-neutralizing antibodies.<sup>136</sup> These various antibodies arise at different rates and times with respect to the onset of the illness and persist for varying lengths of time. Thus anti-VCA antibodies rise early, reach peak titers within a week or two after the onset of the illness, and persist for years if not for life.<sup>136</sup> But, while the presence of anti-VCA

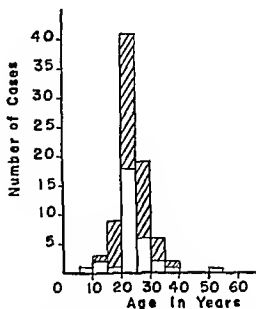


Fig 43-1 Age and sex of 82 patients with infectious mononucleosis at the Johns Hopkins Hospital. Males are indicated by hatched columns, females by open columns. All the patients were white.

antibodies is an excellent indicator of infection, immunity appears to be mediated by virus-neutralizing antibodies.<sup>135</sup> These antibodies appear later and rise at a slower rate; peak levels, which may persist for years or life, may not be reached until the sixth or seventh week.<sup>138</sup> Anti-ID (anti-EA) antibodies are only found in 70% of patients with infectious mononucleosis, probably because they appear later than the anti-VCA antibody and fail to persist.<sup>136</sup> (2) Typical heterophil-positive infectious mononucleosis only occurs in individuals without previous significant anti-EBV titers and never in those with pre-existing anti-EBV antibodies.<sup>83,139,205,216,285</sup> (3) When long-term lymphoblastoid cell cultures are successfully established, they almost *always* contain the EB virus, whether they have been derived from patients with Burkitt's lymphoma, infectious mononucleosis, or leukemia, or, under special circumstances, from healthy individuals.<sup>192</sup> The ease of establishing such cultures from persons with a recent or past history of infectious mononucleosis<sup>67,137,192</sup> contrasts markedly with the difficulty en-

countered with cells of individuals without such a history and has been used as evidence for the role of the EBV in infectious mononucleosis.<sup>192,267</sup> (4) The isolation of a virus from the throat washings of patients with infectious mononucleosis has been reported,<sup>115</sup> and filtrates of throat washings from these patients have been found to contain a herpes-like virus<sup>225</sup> that as yet has not been definitively identified. Such filtrates transformed cord blood lymphocytes.<sup>43,193,225</sup> However, transformation experiments must be viewed with some caution because the EBV viral genome was shown to persist in an EBV-negative cell line derived from a patient with Burkitt's lymphoma.<sup>127</sup> (5) The transmission of infectious mononucleosis by EBV-infected material or by purified virus would greatly strengthen the etiologic argument for the EB virus.<sup>267</sup> Two such instances of infectious mononucleosis transmission have been reported, but the details have not been published.<sup>115</sup> Transmission of EB virus infections through blood transfusions has been documented more frequently<sup>17,106,131</sup> and has sometimes been accompanied by typical clinical and laboratory manifestations of infectious mononucleosis.

In summary, there is strong evidence for the role of the EB virus in typical *heterophil-positive* infectious mononucleosis, although the etiologic relationship has not been established to the complete satisfaction of all workers.<sup>110,199,267</sup> *Heterophil-negative* infectious mononucleosis may be associated with EB virus infections,<sup>83,169</sup> especially in children,<sup>83</sup> but more commonly has been associated with cytomegalovirus infections.<sup>167,169</sup> Occasionally heterophil-negative infectious mononucleosis has been described in association with rising titers to herpes simplex and rubella viruses.<sup>285</sup>

### Epidemiology

**AGE.** Infectious mononucleosis occurs most frequently in young adults, the peak incidence being in persons between the ages of 17 and 25 years<sup>9,143</sup> (Fig. 43-1). The

**Table 43-1. Symptoms and Signs of Infectious Mononucleosis**

<i>Symptoms</i>	<i>%</i>	<i>Signs</i>	<i>%</i>
<i>Malaise and fatigue</i>	90-100	<i>Adenopathy</i>	100
<i>Sweats</i>	80-95	<i>Fever</i>	80-95
<i>Sore throat, dysphagia</i>	80-85	<i>Pharyngitis</i>	65-85
<i>Anorexia</i>	50-80	<i>Splenomegaly</i>	50-60
<i>Nausea</i>	50-70	<i>Bradycardia</i>	35-50
<i>Headache</i>	40-70	<i>Periorbital edema</i>	25-40
<i>Chills</i>	40-60	<i>Palatal enanthem</i>	25-35
<i>Cough (mild)</i>	30-50	<i>Liver or splenic tenderness</i>	15-30
<i>Myalgia</i>	12-30	<i>Hepatomegaly</i>	15-25
<i>Ocular muscle pain</i>	10-20	<i>Rhinitis</i>	10-25
<i>Chest pain</i>	5-20	<i>Jaundice</i>	5-10
<i>Arthralgia</i>	5-10	<i>Skin rash</i>	3-6
<i>Diarrhea or soft stools</i>	5-10	<i>Conjunctivitis</i>	5
<i>Photophobia</i>	5-10	<i>Pneumonitis</i>	3
<i>Abdominal pain</i>	5		
<i>Epistaxis</i>	3		

(From Finch <sup>90</sup> courtesy of the author and Blackwell Scientific Publications.)

disease is sufficiently uncommon in individuals beyond 30 years of age and so rare in those over 40 that the diagnosis in persons in the middle or late years should be made with special care, although it cannot be excluded on the basis of age alone.<sup>117,143,252</sup>

**SEX.** Statistics on sex distribution vary considerably,<sup>9,13,70</sup> but there is probably no sex predilection.<sup>12,143</sup>

**RACE.** While typical infectious mononucleosis is seen in American Negroes, the incidence of the disease appears to be lower than in Caucasians,<sup>126,143</sup> especially among army personnel; of 234 officers and enlisted men with infectious mononucleosis in Hoagland's series only 2 were black, while blacks constituted 12 to 14% of all army personnel.<sup>143</sup>

**SEASONAL INCIDENCE.** There is little evidence for a seasonal incidence,<sup>12,46,143</sup> but the disease appears to be most frequent after college vacations.<sup>12</sup> Epidemics are said to appear in the spring,<sup>15</sup> but Hoagland's careful analysis<sup>143</sup> suggested that, in most reported epidemics,<sup>193,208,276,292</sup> the diagnosis of infectious mononucleosis was not well established.

**MODE OF TRANSMISSION.** The contagiousness of infectious mononucleosis is

quite low and it is exceptional for members of a patient's family, roommates of the same sex, and other close associates to develop clinically obvious infection.<sup>12,46,143,210</sup> Nevertheless, the incidence of hematologic changes may be higher in contacts than in a control group,<sup>223</sup> and anti-EBV titers may rise in about 20% of susceptible contacts.<sup>210</sup> Most authorities agree that infectious mononucleosis is usually transmitted by the intimate oral exchange of saliva as occurs in kissing of more than filial intensity; it is sometimes transmitted by rapid, indirect oral means such as passing a bottle from mouth to mouth, and it is only rarely transmitted by blood transfusions.<sup>12,143</sup>

Clinical data suggest that acute infections are usually acquired from an infectious mononucleosis carrier: such carrier states have now been demonstrated.<sup>193</sup> Most infections are not acquired from a sick individual and subclinical mononucleosis, though frequently postulated, is uncommon.<sup>143</sup>

#### Clinical Manifestations (Table 43-1)

In the absence of a firmly established etiologic agent, various students of this disease in the past allowed themselves more or less liberty in its definition. Thus, many have held

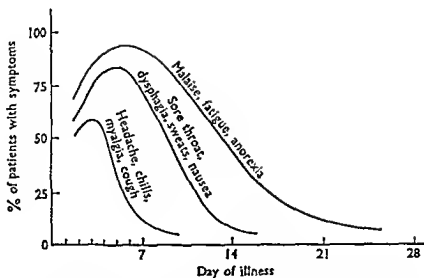


Fig. 43-2. Usual frequency and duration of major symptoms in young adults with infectious mononucleosis (From Finch,<sup>90</sup> courtesy of the author and Blackwell Scientific Publications)

that the manifestations of infectious mononucleosis are protean<sup>15</sup> and that a great variety of disorders may be simulated by this condition. Some would make the diagnosis in the absence of a positive heterophil antibody response or even in the presence of only equivocal morphologic changes in the blood, basing their diagnosis on the presence of more or less typical clinical findings or even on little more than the lack of any better explanation for a self-limited febrile disorder in a young person. Others,<sup>110</sup> on the other hand, would restrict the diagnosis to patients in whom lymphocytes constitute more than 50% of the leukocytes, in whom "atypical" lymphocytes are present, and in whom both of these features have been present over a period of at least 10 days. When the diagnosis was restricted to patients displaying these laboratory features in addition to a heterophil antibody titer of at least 1:28 after guinea pig absorption, Hoagland found the clinical picture to be very consistent and not protean.

**SYMPTOMS** (Figure 43-2). The *incubation period* is uncertain and may be as long as 33 to 49 days.<sup>140</sup> In most young adults the symptoms are of fairly abrupt onset, although

close questioning will frequently elicit vague complaints of lassitude and ill-being for several days before the onset of more pronounced symptoms. Most of the early symptoms are nonspecific in nature. Excessive fatigue and general malaise may be accompanied by a sensation of feverishness, sweating, and chills; severe rigors almost never occur and the patterns of fever and perspiration are moderate and nonspecific.

*Anorexia* is a common early symptom. Its intensity and duration are often linked to the severity of sore throat and dysphagia. The anorexia frequently persists into the second and third week of illness. *Nausea* is equally common but vomiting is rare. Smokers often develop an acute distaste for cigarettes, and smoking may precipitate attacks of nausea. This may be one of the earliest symptoms of infectious mononucleosis. Other gastrointestinal complaints are rare, but some patients experience vague abdominal distress, including a peculiar sensation in the left upper quadrant when they are running, rolling over in bed, or are jarred. A small number of patients have several soft stools daily, but actual diarrhea is uncommon.

*Sore throat and dysphagia* are among the most important manifestations of infectious

mononucleosis and occur in 80 to 85% of all the patients; in some young adults, sore throat may be the only symptom of the disease. It usually develops gradually during the first week of illness and subsides during the second week. Occasionally sore throat returns later because of a superimposed beta hemolytic streptococcal infection. In the majority of patients the symptoms of pharyngitis are mild, but in some even taking sips of water may be painful. Rarely, massive tonsillar and pharyngeal edema may cause virtually complete pharyngeal obstruction.

*Headaches* occur early and usually subside during the first week; they may be retro-orbital in location. A few patients complain of photophobia and ocular muscle pain and some particularly observant individuals have complained of puffiness of the eyelids. Severe cough and chest pain are relatively rare symptoms, although mild cough is more commonly present. A few patients complain of myalgia, but the symptoms are usually mild and are confined to the neck and upper back.

**SIGNS (Fig. 43-3).** *Lymph node enlargement* is invariably present at some time and is bilateral<sup>140</sup>; the cervical glands were always enlarged in two large series of subjects.<sup>46,140</sup> Adenopathy increases rapidly during the first

week of illness, remains stationary during the second, and then slowly wanes. Anterior cervical enlargement is usually present. The posterior cervical nodes may be enlarged and are often palpable down to the clavicle,<sup>143</sup> but this finding is not pathognomonic of infectious mononucleosis. Enlargement of axillary and inguinal nodes is usually, but not invariably, present. Roentgenologically detectable hilar adenopathy is very rare (<1%) and is usually mild. Heat, redness or marked tenderness, fluctuation, and suppuration are conspicuous by their absence and militate against a diagnosis of infectious mononucleosis.

One would expect the *spleen* to be enlarged in all these patients, but the enlargement need not be palpable. Since splenomegaly has been looked for with varying degrees of diligence, it is difficult to be sure of the exact incidence of palpable enlargement. Nevertheless, during the second week of the illness the spleen is palpable in at least half to three quarters of all the patients.<sup>90,143</sup> Generally it extends 2 to 3 cm below the costal margin, but occasionally it may be larger and may even reach the iliac crest.<sup>15</sup>

Slight *hepatomegaly* is detected in 15 to 25% of patients with infectious mononucleosis. This may be accompanied by percussion tenderness over the liver and discomfort on

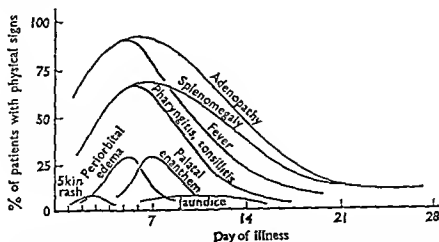


Fig. 43-3. Usual frequency and duration of major physical signs in young adults with infectious mononucleosis. (From Finch,<sup>90</sup> courtesy of the author and Blackwell Scientific Publications.)

palpation. The course of hepatomegaly closely follows that of adenopathy and splenomegaly. Jaundice may develop in about 10% of the patients and usually reaches its peak in the second week of the illness. Liver biopsy has shown varying degrees of sinusoidal infiltration, in rough agreement with functional tests<sup>162</sup> (see below).

The fever is of no characteristic type. It may be very transient and slight in degree, but in as many as one third of the patients it reaches a peak of 103° to 104°F.<sup>15,140</sup> This may occur at the end of four to eight days, the rise taking place in a remittent manner. A secondary rise after an initial drop to normal may accompany the onset of glandular swelling or sore throat. In one series of 196 cases, 11% of all the patients had no fever.<sup>46</sup> In another group of 200 cases, fever was present in all but five patients.<sup>140</sup> In the most seriously ill patient whom the authors have seen, a temperature of 106.6°F was reached following a chill and levels of 104°F were attained on three other days. This patient's throat was comparatively normal in appearance. The temperature curve may, occasionally, suggest malaria. In some instances it may suggest typhoid fever, especially since the pulse may be relatively slow.

*Pharyngeal inflammation* usually appears in the first week and then subsides rapidly. In about 25% of the patients, pharyngitis occurs first after the initial week of illness. It varies in intensity, but hyperplasia of the pharyngeal lymphoid tissue is almost always present. The palatal arch and uvula often have a gelatinous appearance, but significant edema of the uvula is unusual. Exudate, usually spotty, is seen in about a third of the patients.<sup>143</sup>

A *palatal exanthem*, consisting of 5 to 20 round, sharply defined, red spots at the junction of the soft and hard palates, is of great diagnostic, though not pathognomonic, value in infectious mononucleosis.<sup>140,143</sup> The spots occur in the second week of illness, turn brown within 48 hours, and disappear four to five days later.

*Edema of the eyelids*<sup>220</sup> has been described in a third of all subjects.<sup>140</sup> This consists of drooping of the swollen orbital portion of the

upper eyelids upon the palpebral portion and sagging of the latter. As a result the ocular aperture is narrowed.

A small number of patients develop a fine macular rash,<sup>70,143</sup> but in many the rash is probably due to drug allergy.<sup>143</sup> Indeed, several observers have noted that patients suffering from infectious mononucleosis were peculiarly prone to develop rashes when treated with ampicillin.<sup>21,228,246</sup> In one such study<sup>290</sup> the incidence of skin eruptions in the absence of antibiotics was 1%, with penicillin 3%, and with ampicillin 50%!

**COMPLICATIONS.** Physicians caring for large numbers of patients with infectious mononucleosis soon become impressed by the monotonous regularity with which the few basic signs and symptoms recur. Weird clinical patterns and serious complications are probably seen in no more than 1% of all patients.<sup>90,143</sup> Misconceptions about a higher incidence stem from the fact that most of the medical literature on infectious mononucleosis deals with complications and from impressions of sub-specialists who frequently do not see patients with uncomplicated cases. The most important complications reported in the literature are listed in Table 43-2.

One of the commonest of the rare complications is *splenic rupture*, which has led to death in several cases<sup>143,221</sup> and may occur with an incidence of 0.2% or higher. The diagnosis of a ruptured spleen should be entertained whenever a patient with infectious mononucleosis has severe or even moderate pain below the left costal margin, especially if the pain is accompanied by radiation to the left shoulder or evidence of impending peripheral vascular collapse. Other signs include those characteristic of peritoneal irritation, abdominal tenderness, and shifting dullness if massive intra-abdominal bleeding has occurred. Tachycardia is an extremely useful sign since bradycardia is the rule in uncomplicated infectious mononucleosis and rates exceeding 100/minute are rare. Frank rupture may be preceded by one or more episodes of subcapsular hemorrhage or minor capsular tears, which may be difficult to

differentiate from rupture and, since potentially fatal bleeding may occur at any time, some authorities recommend surgical treatment whenever the typical pain pattern is accompanied by increasing tachycardia<sup>143</sup>, rupture with obvious hemorrhage *always* makes surgical therapy mandatory. Delayed surgical intervention is often attributable to the misconception that abdominal pain is a common feature of the uncomplicated disease.<sup>143</sup>

**Cardiac complications** are rare. Electrocardiographic abnormalities, especially non-specific T-wave changes, are the most common and may occur in about 10% of the patients.<sup>70,142</sup> Pericarditis has been reported,<sup>239,253,255</sup> but is very rare; whenever it is found, other causes should also be sought.

**Neurologic manifestations** may occur in 1 to 2% of the patients<sup>143,248</sup> and range from asymptomatic pleocytosis, increased protein in the cerebrospinal fluid, or electroencephalographic abnormalities (which may be found in as many as a third of all subjects) to occasional instances of life-threatening involvement of the nervous system. When serious neurologic complications occur the mortality rate may be as high as 8 to 11%,<sup>128,176</sup> and, in addition, the incidence of serious residual damage may be as high as 12%.<sup>128</sup> Reported neurologic complications are listed in Table 43-2.

The most serious respiratory complication is *acute airway obstruction*, which is usually due to extreme hyperplasia of the tonsils and other pharyngeal lymphatic tissue. Pleural effusion and pulmonary parenchymal changes are exceedingly rare.

Evidence for some degree of *hepatic involvement* is found in the great majority of patients, but is usually mild, and the necrosis and inflammatory exudate that are seen in infectious hepatitis are rarely encountered.<sup>140</sup> Liver failure is exceedingly rare.<sup>224</sup> *Pancreatitis* is an equally rare complication.<sup>86,200,298</sup>

The most common hematologic complications are hemolytic anemia<sup>62,65,113,304</sup> and thrombocytopenia.<sup>38,45,143,232</sup> The incidence of hemolytic anemia may be 3%. Hemolysis

**Table 43-2. Complications of Infectious Mononucleosis**

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1	Splenic rupture <sup>90, 143, 145, 224, 233</sup>
2	Cardiac complications ECG changes <sup>70, 142</sup> Pericarditis <sup>239, 253, 255</sup> ? Myocarditis <sup>222</sup>
3	Neurologic complications <sup>128, 176, 224, 248, 292</sup> CSF abnormalities <sup>16, 19, 222, 255, 311</sup> EEG abnormalities <sup>222, 248</sup> Mononeuritis <sup>85, 104, 143, 248</sup> Facial nerve palsies <sup>85, 149</sup> Guillain-Barré Syndrome <sup>74, 255</sup> Myelitis <sup>143, 248</sup> Meningitis <sup>104, 128, 143, 255</sup> Meningoencephalitis <sup>18, 248</sup> Encephalitis <sup>85, 104, 255, 269</sup> Psychiatric disorders <sup>143, 163, 173</sup>
4	Respiratory complications Acute airway obstruction <sup>121, 224</sup> Pulmonary parenchymal changes <sup>187</sup> Pleural effusion <sup>75</sup>
5	Hematologic complications Hemolytic anemia <sup>62, 65, 113</sup> Hypoplastic anemia <sup>304</sup> Thrombocytopenia <sup>38, 45, 232</sup> ? Granulocytopenia <sup>92, 199, 305</sup>
6	Liver Failure <sup>224</sup> Pancreatitis <sup>86, 200, 298</sup>
7	Death <sup>224</sup>

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usually is mild; in about 70% of the patients with hemolytic anemia the reaction to Coombs' test is positive and in about the same number the titers of cold agglutinins are increased.<sup>304</sup> Sometimes, however, there is no detectable antibody of any kind. The cold agglutinins are specific for the red cell antigen i,<sup>148,240</sup> (see page 458) but, since a similar incidence of anti-i antibodies (70%) also is found in patients without hemolysis,<sup>148,240</sup> the role of these antibodies in red cell destruction is unclear. The antibody reported in these studies had the characteristics of an IgM molecule, but others have also found IgG anti-i antibodies in the sera of 90% of patients with infectious mononucleosis.<sup>35</sup> The latter incomplete antibody only caused agglutination in the presence of anti-IgG antibodies. Thus, since anti-i antibodies are commonly seen but hemolytic anemia is rare, it is doubtful whether they are the cause

of hemolysis in all patients who have this antibody. In only a few cases has hemolysis been shown to be clearly due to anti-I.<sup>33,35,148,155,279</sup> One case of hemolysis due to Donath-Landsteiner cold hemolysins has been reported.<sup>293a</sup>

In some instances, infectious mononucleosis may have accelerated hemolysis in patients with underlying hereditary spherocytosis<sup>65,113</sup> or thalassemia.<sup>274</sup>

*Thrombocytopenic purpura* is a very rare complication<sup>15,143,232</sup> although mild depression of the platelet count ( $100$  to  $140 \times 10^9/l$ ) may be found in perhaps one half of the patients,<sup>38</sup> usually early in the disease. When thrombocytopenic purpura does occur, most of its subjects seem to be children.<sup>143</sup> As a rule, the purpura develops about a week after the appearance of the first symptoms of infectious mononucleosis and the bleeding manifestations last about 10 days, although some degree of thrombocytopenia persists for a month.

Serious suppression of marrow function is rare, but fatal aplastic anemia was noted in one patient six weeks after what appeared to be typical infectious mononucleosis.<sup>304</sup> The sequence of events in this patient was not unlike that observed in young males who develop fatal marrow aplasia while recovering from infectious hepatitis.<sup>180</sup>

*Granulocytopenia* has been reported in rare instances<sup>82,170a,199,305</sup> but the possibility of other more common causes of granulocytopenia such as drugs could not be excluded in some of the cases.

## Laboratory Findings

### Blood Cells

The characteristic feature of infectious mononucleosis is the polymorphism of the mononuclear cells found in the blood. In addition to normal lymphocytes and monocytes, large mononuclear cells that usually are not present in the blood are observed (Plate XVII). Although not specific,<sup>69</sup> since they

may be found in the blood of patients with viral infections or with a number of other conditions (see below),<sup>303</sup> they form a prominent feature of infectious mononucleosis.

The abnormal cells vary greatly in size and shape. They possess a nucleus that may be oval, kidney-shaped, or slightly lobulated and cytoplasm that most frequently is nongranular and vacuolated or foamy in appearance. The nuclear chromatin forms a coarse network of strands and masses and is not clearly differentiated from the parachromatin. The identity of these cells has been disputed, but most hematologists regard them as highly differentiated, mature lymphocytes.<sup>41,68</sup> They do not show granules when stained for peroxidase and leukocyte alkaline phosphatase, and can thus be distinguished from cells of the granulocytic series and, to some extent, from monocytes.

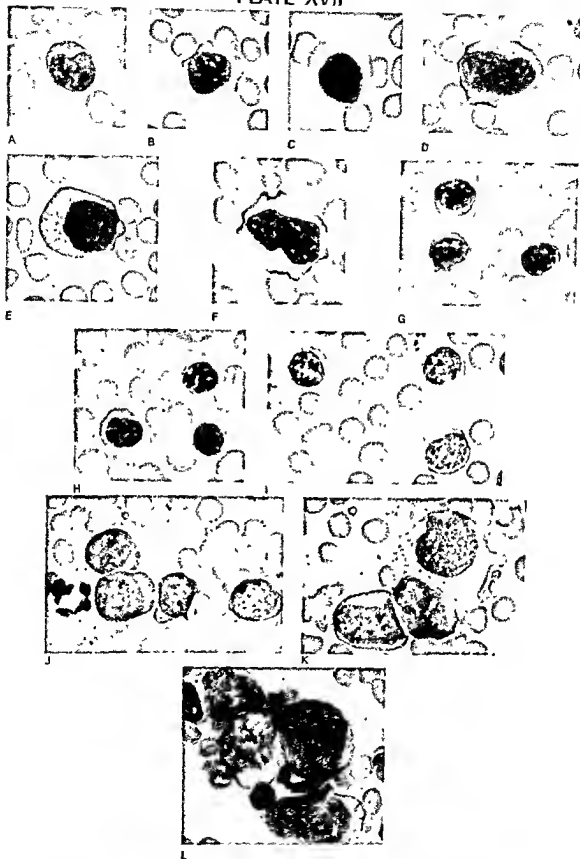
These "leukocytoid lymphocytes," the form most frequently seen, were classed as Type I by Downey,<sup>68</sup> who described two other types of abnormal lymphocytes in the blood of infectious mononucleosis patients. Compared with Type I cells, Type II cells are less varied but larger, their nuclear chromatin is not so condensed, and their cytoplasm is more homogeneous and not so basophilic or so vacuolated. Type III cells resemble Type I cells but show some leukemic features. Their nuclei show a diffuse sieve-like chromatin and possess one or two nucleoli. Their cytoplasm is vacuolated and may be quite basophilic. Excellent illustrations of these cells were published by Downey and McKinley.<sup>68</sup>

Infectious mononucleosis cells obtained directly from the blood appear to be T cells (page 295)<sup>252a</sup> and may be produced in the thymus-dependent areas of lymph nodes and spleen, since these areas show a great deal of proliferative activity.<sup>78a</sup> In vitro these cells proliferate actively and there is evidence for increased DNA and RNA synthesis, especially early in the disease.<sup>39,78,185</sup> Such cells also have been studied by histochemical methods<sup>100</sup> and with the electron microscope.<sup>218,236</sup>

In contrast, long-term suspension cultures



# PLATE XVII



*Infectious mononucleosis, chronic lymphocytic leukemia, and non-Hodgkin's lymphoma (blood and bone marrow, Wright's stain  $\times 1000$ )*

A-F, Infectious mononucleosis. A, Downey, type I, B and C, Downey type II, D, E, F, Downey type III  
 G, H, I, Chronic lymphocytic leukemia, showing the characteristic cells seen in the blood

J, K, L, Non-Hodgkin's lymphoma cells in bone marrow. J, lymphosarcoma, poorly differentiated (lymphoblastic)

**HETEROPHIL ANTIBODIES** A serologic diagnostic test for infectious mononucleosis has been available since Paul and associates<sup>221</sup> discovered that the sera of patients with infectious mononucleosis contain agglutinins against sheep red cells. They called the antibody "heterophil," since it reacted with an heterologous antigen that obviously had not elicited its production. Most authorities consider a positive response to the heterophil antibody test to be a *sine qua non* in the diagnosis of infectious mononucleosis.<sup>140,143</sup> It was soon learned that the antibody reacted not only with sheep red cells but also with beef and horse erythrocytes,<sup>4,8</sup> although in different reaction patterns, the mechanism of which is poorly understood<sup>267</sup>; while sheep and horse erythrocytes are readily agglutinated by heterophil antibodies, they are not lysed, whereas bovine red cells are readily lysed, but not agglutinated.<sup>57,175,277,294</sup>

The heterophil antibody is an IgM globulin<sup>41,171,268,291,310</sup> which in some studies<sup>309</sup> was shown to have lambda light chains only. In spite of this relatively homogeneous structure, there seems to be some heterogeneity of antigenic specificity; it has been shown, for instance, that absorption of infectious mononucleosis sera with bovine red cells removes all activity against sheep and horse cells as well, while absorption with sheep cells removes only part of the activity against equine and bovine cells.<sup>245,268,277,294</sup> Thus bovine cells have some antigenic specificities not found on cells from these other sources, and infectious mononucleosis sera contain antibodies against several antigenic specificities.

Our knowledge of the nature of the heterophil antigen is very fragmentary. The antigen has been shown to be thermostable<sup>4</sup> in contrast to the Forssman antigen. Its immunologically reactive sites appear to be carbohydrate in nature, since they are completely destroyed by neuraminidase, but retain their antigenicity in the presence of proteases.<sup>258</sup> When heterophil receptors were extracted from horse erythrocytes with hot 75% ethanol,<sup>93</sup> the material was found to have a carbohydrate content of 40% with a hexose:hexosamine:sialic acid ratio of 1:1:1. The

peptide portion was greatly enriched in acidic and hydroxylamino acids, which accounted for 43% of all amino acids. As in studies with whole red cell preparations (see above), extracts of bovine origin appeared to have some antigenic determinants not shared with sheep and horse red cells.<sup>93</sup> In addition, the bovine antigen has less sialic acid and carbohydrate and more nitrogen than the other preparations, and retains some infectious mononucleosis reactivity after treatment with neuraminidase.<sup>93</sup>

The carbohydrate nature of the antigen probably explains the observation that the antibody response is almost exclusively IgM, as are the antibody responses to other carbohydrate antigens such as certain blood group substances and the salmonella O antigen.<sup>171</sup>

The heterophil antibody test is carried out as follows.<sup>36</sup> The patient's serum is heated for 30 minutes at 56°C to destroy complement. Only 0.1 ml of a 2% suspension of sheep red corpuscles is needed for the test. The cells should not be less than 24 hours old, nor more than a week old. They must be washed three times in physiologic saline solution on the day of the test, mixing them each time with two to three times as much physiologic solution of sodium chloride. The third centrifugation should concentrate the cells to about half their original volume. The supernatant fluid must be clear after the third centrifugation.

Serial dilutions of serum ranging from 1:7 to 1:7168 are mixed with equal volumes of the 2% red cell suspension. A control tube contains saline solution instead of serum. After shaking, the test tubes are allowed to stand at room temperature for two hours. They are then shaken again until the sediment has been suspended. Agglutination of the corpuscles indicates the presence of heterophil antibody in that tube. The highest dilution in which this can be detected with the naked eye, or with the lowpower microscope objective, is taken as the end point.

Antisheep agglutinins are present in titers up to 1:28 in most normal persons, and occasionally even in a titer of 1:56.<sup>57</sup> In various infections a titer of 1:112 and occasionally

Table 43-3. *Differential Test for Infectious Mononucleosis*

Serum Derived from	Absorption of Antisheep Agglutinins by	
	Guinea Pig Kidney	Beef Red Cells
Persons without serum disease or infectious mononucleosis	+	±
Serum sickness	+	+
Infectious mononucleosis	-	+

+ indicates complete absorption  
 - indicates incomplete absorption  
 ± indicates complete or partial absorption

of 1:224 may be obtained. Persons receiving injections of horse serum and horse immune serum may develop titers as high as any found in infectious mononucleosis. After various kinds of known or unknown antigenic stimulation a titer of 1:448 and higher is occasionally noted.

For these reasons the test for heterophil antibodies has been called *presumptive*.<sup>58</sup> In the presence of clinical and hematologic findings suggestive of infectious mononucleosis, a titer of 1:224 or higher can be interpreted as confirming the diagnosis. In infectious mononucleosis, positive heterophil reactions almost always appear during the first two weeks of the illness.<sup>140</sup> Highest titers are usually found during the second and third weeks. As a rule, positive reactions last four to eight weeks. They have been observed to persist for as short a time as seven to nine days or for as long as 18 weeks.<sup>57</sup> The titer bears no relation to the severity of the disease or to the leukocyte changes. There are wide variations in the agglutinability of erythrocytes from different sheep.

If the titer of the presumptive test is less than 1:224 in the presence of clinical and hematologic findings suggestive of infectious mononucleosis, if the titer is 1:224 or higher in the absence of such findings, or if the patient gives a history of a recent horse serum injection, the results should be checked by a differential test.

The *differential test*<sup>58</sup> is based on the observation that heterophil antibodies in normal serum, in horse serum sensitization, and in

a variety of infections can be absorbed completely by Forssman antigen (guinea pig or horse kidney), but the antibodies present in the serum of patients with infectious mononucleosis are not completely absorbed. On the other hand, absorption with a suspension of beef cells removes the anti-sheep agglutinins completely in infectious mononucleosis, but may leave some antibody in other sera. It is the combination of (1) no or incomplete removal of antibodies with Forssman antigen and (2) complete removal with beef cells (Table 43-3), which is characteristic for infectious mononucleosis. The original differential test offers a very high degree of specificity.<sup>57</sup> A simple capillary screening test has also been devised.<sup>174</sup>

Elevated titers of hemolysin for beef erythrocytes are present in infectious mononucleosis. This forms the basis of another satisfactory test for the disease.<sup>191</sup>

Papain and other plant proteases have been found to remove specifically the receptor for anti-infectious mononucleosis antibodies on sheep erythrocytes without inactivating the receptor for the Forssman and serum sickness antibodies. This is the principle of still another useful diagnostic test<sup>259,300,301</sup> including a simple slide test.<sup>182</sup>

Perhaps one of the easiest and yet most specific tests devised for the diagnosis of infectious mononucleosis is a slide test using formalinized horse red cells as an immunologic indicator.<sup>55,59,147,278</sup> The cells are agglutinated only by the heterophil antibody of infectious mononucleosis and not by any of the

others. This test has many advantages: it seems to be specific, the reagents are available commercially and stable, and the test is easily and rapidly performed in the physician's office. The diagnostic accuracy of the test has been found to be very high (99%),<sup>117</sup> but, since the red cells tend to lose some agglutinability in the process of formalin treatment, the test tends to miss a few low titer sera.<sup>102,175</sup>

Lee and associates have developed a test in which absorptions are performed with the classic combination of guinea pig kidney and boiled ox erythrocytes.<sup>175</sup> The speed of absorption is increased by using very fine suspensions of guinea pig kidney and red cell stromata rather than whole beef red cells. The sensitivity of the test was increased by using horse erythrocytes which are agglutinated to a higher titer than sheep erythrocytes.<sup>175</sup> This test ("Monospot") is probably the most reliable slide test available.<sup>267</sup>

**OTHER ANTIBODIES.** In addition to the heterophil antibody, several other antibodies are found in the sera of patients with infectious mononucleosis. These include cold reactive anti-i antibodies, Donath-Landsteiner cold hemolysins (see page 1363), and an antibody against Rhesus monkey red cells as well as lymphocytotoxins, antibodies directed against specific populations of T and B cells, and smooth muscle antibodies.<sup>76a,212</sup>

Antinuclear antibodies also have been found in the sera of some infectious mononucleosis patients.<sup>77,160</sup> Other patients were found to have a falsely positive reaction to the VDRL test for syphilis, but, according to some authorities, the response to this test may often have been truly positive, since many of the early reports originated during World War II when a true positive VDRL reaction was not rare among young adults.<sup>141</sup>

The sera of some infectious mononucleosis patients also contain *cryoglobulins*.<sup>158,159</sup> The cryoprecipitate consists of IgG and IgM, although IgA may be coprecipitated. Heterophil antibodies, cold agglutinins, rheumatoid factor, antinuclear antibodies, and syphilitic reagins have been reported in connection

with these cryoprecipitates.<sup>158,159</sup> Cold reactive rheumatoid factors have been detected in the sera of over half of the infectious mononucleosis patients.<sup>36,111</sup>

Since so many antibodies have been linked with the development of infectious mononucleosis, it is not surprising that immunoglobulin levels are elevated in this disease.<sup>1,302</sup> While this is especially true for IgM globulins, lesser elevations have also been seen in IgG and IgA globulins. The pattern of changes is similar to that seen in the normal immune response: IgM is elevated first and IgG later.<sup>175</sup> Antibodies with recognized antigenic specificities such as the heterophil antibody only make up a small proportion of the total gamma globulins of the serum<sup>179,302</sup> and the antigenic specificity of the major portion of the increased gamma globulins remains unknown.

### Other Laboratory Findings

Many patients with infectious mononucleosis show mild to moderate abnormal responses to *liver function tests*.<sup>61,143</sup> The reported incidence has varied from about 40 to 100%, depending on the severity of the disease, the time of testing, and the diligence with which the changes were sought. Reported enzyme changes include elevations of isocitric dehydrogenase, alkaline phosphatase, lactic dehydrogenase, glutamic pyruvate transaminase, phosphohexose isomerase, and aldolase, in roughly that order of frequency. Positive reactions to flocculation tests are common and response to the BSP retention test may also be abnormal. Perhaps a third of the patients have mild to moderate elevations of serum bilirubin, but levels above 8 mg% are exceedingly rare.<sup>143</sup> Dissociation between serum alkaline phosphatase and serum bilirubin values has been reported,<sup>6,254</sup> but the practical value of this observation is doubtful. Because manifestations of impaired liver function tend to rise and fall concomitantly and are most pronounced by the middle or end of the second week of the infection (Fig. 43-4), it is unnecessary to order batteries of tests.

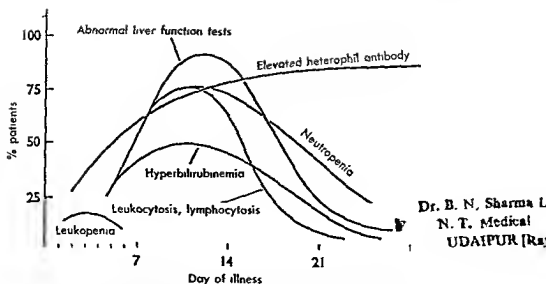


Fig 43-4 Major laboratory findings in adults with infectious mononucleosis (From Finch,<sup>91</sup> courtesy of the author and Blackwell Scientific Publications)

The urine usually is normal. Occasionally, proteinuria or hematuria is present. Mild pyuria also has been noted. In many cases the described abnormalities are not related to infectious mononucleosis *per se*. Renal function is unimpaired. When the patient is jaundiced, bile and increased concentrations of urobilinogen may be found.

The cerebrospinal fluid pressure may be elevated, and there may be pleocytosis and increased levels of protein. The sugar content is normal. In a few of the patients, heterophil antibodies have been demonstrated in the CSF.<sup>90</sup>

### Differential Diagnosis

The diagnosis of infectious mononucleosis usually presents no problem. In most instances a young adult complains of malaise, fatigue, anorexia, and a sore throat, and is found to have fever, adenopathy, pharyngitis, and splenomegaly. Examination of the blood and, above all, a heterophil antibody test and/or a rising EBV titer confirm the diagnosis. Complications are very rare and signs of significant respiratory, cardiovascular, intestinal, urinary, or joint disease make consideration of other diagnoses mandatory. Other manifestations, such as a nasal dis-

charge or congestion, painful or extremely tender nodes, watery diarrhea, and signs suggestive of acute appendicitis, are so rare in infectious mononucleosis as to militate against this diagnosis.<sup>140</sup> Sometimes the characteristic clinical findings of infectious mononucleosis are not apparent until the end of the second week of illness, and heterophil antibodies may not be detectable for a week or so.

The *pharyngitis* of infectious mononucleosis must be differentiated from acute streptococcal pharyngitis, diphtheria, and acute viral pharyngitis of other types. The *fever and general malaise* may suggest one of the salmonella infections, listeriosis, brucellosis, subacute bacterial endocarditis, the lymphadenopathic form of toxoplasmosis, or malaria. Drug fever and serum sickness-like reactions may also suggest infectious mononucleosis, since they are often characterized by fever, jaundice, lymph node enlargement, and atypical lymphocytes. Clinical features should suffice to differentiate the *lymph node enlargement* of infectious mononucleosis from that occurring regularly in Hodgkin's disease and in lymphosarcoma, and that which may be seen with neoplastic metastases and inflammatory disease. Generalized lymph node enlargement, including the

nodes in the postauricular and occipital areas, is also characteristic of rubella, however. In addition, a palatine exanthem may be present in as many as a third of all patients with rubella<sup>113</sup> and atypical lymphocytes have been described.

The hematologist is most frequently called upon to differentiate infectious mononucleosis from other hematologic diseases, especially acute leukemia, but the latter diagnosis should never be made in the absence of other hematologic manifestations such as anemia, thrombocytopenia, and typical blast cells. Although many leukocytes of patients with infectious mononucleosis appear abnormal, very few contain nucleoli, and thrombocytopenia and anemia are very rare in these patients. It should also be noted that the appearance of the peripheral blood is constantly fluctuating in infectious mononucleosis, whereas this does not happen in leukemia. Bone marrow examination is rarely necessary to differentiate infectious mononucleosis from acute leukemia.

Patients who have suffered from infectious mononucleosis do not have a higher incidence of leukemia, lymphoma, or other malignant conditions subsequently,<sup>193a</sup> but a few cases of concurrent infectious mononucleosis and leukemia have been reported.<sup>61,94,95,204</sup> Indeed, intercurrent infectious mononucleosis has been considered to have a beneficial effect on the survival of patients with acute leukemia.<sup>206</sup> In addition, the sera of all acute leukemia patients with long survivals had higher immune anti-EBV titers than did the sera of control groups. The basis of the beneficial effect of infectious mononucleosis on acute leukemia is unknown and may be nonspecific; the two diseases appear to be etiologically unrelated.<sup>94,95</sup>

*Lymphocytosis*, absolute or relative, may be encountered in a number of diseases other than infectious mononucleosis, as discussed in Chapter 41. These include agranulocytosis, Vincent's angina, tuberculosis, tularemia, pertussis, dengue, mumps, chickenpox, German measles, typhoid fever, infectious hepatitis, serum sickness, and various other allergic states. In some of these conditions, abnormal lymphocytes resembling those of

infectious mononucleosis have been observed, as mentioned earlier. Differentiation from infectious hepatitis sometimes is difficult but reaction to the differential test for heterophil antibodies is negative in the latter.

Marked leukocytosis, due chiefly to the presence of small lymphocytes of normal appearance, and associated neither with splenomegaly, lymphadenopathy, nor a positive heterophil agglutination reaction, suggests acute infectious lymphocytosis (Chapter 41, page 1289).

Atypical lymphocytes have also been described in the post-transfusion syndrome, an infectious mononucleosis-like illness occurring particularly commonly after cardiopulmonary bypass.<sup>157,168,251,256,261</sup> The illness usually occurs one to three months after open heart surgery or after blood transfusions and is characterized by moderate fever, and, in about half the patients, splenomegaly or hepatomegaly. Lethargy and adenopathy are unusual and pharyngitis is absent, thus differentiating this syndrome from infectious mononucleosis on clinical grounds alone. A transient rubelliform rash is occasionally present.<sup>256</sup> The disease is caused by the cytomegalovirus (CMV) and the virologic diagnosis can be confirmed by demonstrating a serial rise of complement-fixing antibodies to CMV. The virus may also be cultured from the patient's blood and tissues. Reactions to tests for heterophil antibodies are characteristically negative and serial increases in anti-EBV titers have not been noted in CMV infections.<sup>261</sup>

Lymphocytosis with atypical cells is the most characteristic laboratory feature of the post-transfusion syndrome, and the peripheral smear may be indistinguishable from that of infectious mononucleosis. The white count usually does not exceed 15.0, but occasionally leukemoid reactions with counts of 35.0 to 75.0  $\times 10^9$  cells/l may be obtained.<sup>157</sup> The bone marrow is said to be normal in appearance. A normochromic normocytic anemia with varying degrees of reticulocytosis is not uncommon and in some instances, at least, appears to be associated with a positive reaction to Coombs' test or with cold agglutinins.<sup>157</sup> Other immunologic "aberrations"

include rheumatoid factors, antinuclear antibodies, and cryoglobulins.<sup>157,168</sup> In most patients with CMV the infection is mild and resolves within a few weeks; in a few the manifestations may last for several months or longer.

### Treatment

There is no specific therapy for infectious mononucleosis. Antibiotics are of no value<sup>249</sup> unless the patient has developed a superimposed streptococcal pharyngitis. Convalescent sera, obtained from patients one or two weeks after they had become free from fever, and given intravenously in total doses of 50 to 300 ml, have been found useful in bringing about symptomatic relief and fall of temperature, and in preventing complications.<sup>172</sup> There is no convincing evidence that chloroquine is beneficial.<sup>309</sup> Adrenocorticosteroids may have dramatic effects on the disease with prompt lysis of fever and visible reduction of lymphatic hyperplasia, usually within 24 hours. Steroids are indicated<sup>2,13,70,143</sup> in patients with hemolytic anemia, thrombocytopenia, and with neurologic complications. They are also recommended for unusually distressing sore throats and when there is incipient airway obstruction. The prolonged use of steroids should be avoided.

Isolation of patients does not seem warranted, since cross infection has not been noted under ordinary circumstances.<sup>15,140</sup> Bed rest need not be enforced and return to normal activities should be governed by the improvement in clinical manifestations of the disease. It has been recommended<sup>143</sup> that patients not travel by automobile for a minimum of three weeks after the onset of symptoms, because of the possibility of a fatal splenic rupture resulting from even rather minor abdominal trauma.

### Prognosis

Infectious mononucleosis is rarely fatal and the prognosis for a complete recovery within two months is virtually 100%<sup>143</sup>; recurrences

of infectious mononucleosis are either extremely rare or nonexistent.

Penman,<sup>224</sup> in reviewing the world literature on infectious mononucleosis, catalogued 87 fatalities attributed to the disease or its complications. However, only 20 of these reports contained adequate evidence for an unequivocal diagnosis of infectious mononucleosis. In the 20 cases, 9 fatalities were due to neurologic complications—four due to respiratory failure from peripheral neuropathy of the Guillain-Barré type and five due to central respiratory paralysis; three were due to splenic rupture and three to secondary infection; other fatal complications included hepatic failure (2 cases) and myocarditis (1 case); in two cases the cause of death was unrelated to infectious mononucleosis.

The actual incidence of fatal complications is difficult to estimate but appears to be less than one per 3,000 cases.<sup>224</sup>

### Pathology

Infectious mononucleosis is a systemic illness whose main pathologic feature is a marked proliferative response within the reticuloendothelial system, especially the lymph nodes and the spleen. Nevertheless, perivascular infiltration by normal and atypical lymphocytes may also occur in many other organs of the body.

The lymph node architecture<sup>48,101,235</sup> may be distorted but is otherwise intact,<sup>101</sup> and this helps to distinguish infectious mononucleosis from malignant lymphomas. The germinal centers are identifiable, but follicular prominence is diminished. This probably is due to the irregular and vaguely defined borders that result from the lymphocytic and reticuloendothelial hyperplasia of perifollicular structures, and especially the medullary cords.<sup>48</sup> In addition, there is focal proliferation of macrophages, and, most characteristically, the presence of "typical" infectious mononucleosis cells throughout the pulp, on the edges of germinal centers, and in the sinuses.<sup>101</sup> Nasopharyngeal lymphoid hyperplasia was consistently found in the autopsy subjects.

The spleen shows striking infiltration of its

fibromuscular structures by mononuclear cells<sup>48, 143</sup>; both the capsule and trabeculae are thin and invaded by proliferating lymphocytes, and thus probably explains the unusual liability to splenic rupture in this disease.

Some enlargement of the liver is frequently seen in patients who come to autopsy.<sup>45</sup> Collections of mononuclear cells are seen throughout the portal areas, throughout the lobules, and within sinuses.<sup>141</sup> Most of these cells are small lymphocytes, but cells corresponding to atypical lymphocytes are also found. Small numbers of eosinophils may be present in the portal areas. Hepatocellular damage is minimal or absent<sup>141, 258</sup> and this feature sharply differentiates infectious mononucleosis from infectious hepatitis. Infectious mononucleosis never leads to cirrhosis of the liver.<sup>143</sup>

The meninges<sup>18</sup> may be congested and edematous and occasional small perivascular hemorrhages are noted. Some patients coming to autopsy have shown evidence of a mild to moderate meningoencephalitis and in some distinct perivascular cuffing is evident within the brain. Changes in the spinal cord appear to be minor, but cellular infiltration of anterior roots has been noted.<sup>18</sup>

Small focal infiltrations of the lungs,<sup>48, 311</sup> kidneys,<sup>48, 311</sup> and myocardium<sup>48</sup> explain some of the minor clinical manifestations of this disorder.

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## Immune Deficiency Diseases

### Definition and Terminology

Infantile Sex-Linked Agammaglobulinemia  
Selective Immunoglobulin Deficiency (IgA)  
Transient Hypogammaglobulinemia of Infancy  
Immunodeficiency with Hyper IgM  
Thymic Hypoplasia  
Immunodeficiency with Ataxia Telangiectasia  
Immunodeficiency with Thrombocytopenia and Eczema  
Severe Combined Immunodeficiency  
Variable Immunodeficiency  
Other Congenital Disorders of Host Defense against Infections  
Acquired Immune Deficiency Syndromes

### Definition and Terminology

Since Bruton's original discovery of agammaglobulinemia in 1952,<sup>5</sup> several thousand patients suffering from a variety of immune deficiency states have been studied in clinics and laboratories throughout the world. It soon became clear that immunologic deficiency can take many forms with a bewildering array of clinical, pathologic, and immunologic findings. Unfortunately, the use of eponyms, inaccurate terminology, and hypothetical pathogenetic mechanisms led to further confusion.

Two broad categories of immune deficiency states—primary and secondary—are now recognized. Primary immune deficiency diseases are genetically determined abnor-

malities of immunologic development that result in defective antibody production or cellular immune responses. Secondary immune deficiencies are due to acquired diseases that interfere with the function of the mature immune system.

While the classification of secondary immune deficiencies presents little difficulty, classification of the primary or inherited defects has generally been unsatisfactory. In an attempt to clarify the categorization the World Health Organization convened a group of investigators who proposed a classification of primary immune deficiencies based on the apparent cellular defect involving stem cells, B-cells, or T-cells (for a discussion of these cells, see Chapter 7) or a combination of these<sup>6</sup> (Table 44-1). Thus, for example, infantile sex-linked agammaglobulinemia is thought to be caused by an almost complete lack of B-cell function, while thymic hypoplasia is the prototype of T-cell defects. Each defect is associated with fairly predictable clinical, pathologic, and immunologic findings, but these may vary somewhat depending on whether the defect is partial (eg, selective immunoglobulin deficiency) or complete (eg, infantile sex-linked agammaglobulinemia) and whether there are other associated defects (eg, hypocalcemia in thymic hypoplasia, thrombocytopenia, and eczema in the Wiskott-Aldrich syndrome). Because of these individual variations it is necessary to consider the major immune deficiency syndromes separately. The terminology proposed by the

Table 44-1. Congenital Immunologic Deficiencies

	Suggested Cellular Defect <sup>a</sup>		
	B-Cells	T-Cells	Stem Cells
1 Infantile sex-linked agammaglobulinemia <sup>4,5,10,12,22</sup>	+		
2 Selective immunoglobulin deficiency (IgA) <sup>14,25,37,38,40,42,43,47,50</sup>	+ (some)		
3 Transient hypogammaglobulinemia of infancy <sup>23,41,42</sup>	+		
4 Immunodeficiency with hyper IgM <sup>44</sup>	+		
5 Thymic hypoplasia (pharyngeal pouch or George syndrome) <sup>48,74,75,76,78</sup>		+	
6 Episodic lymphopenia with lymphocytotoxic <sup>146</sup>		+	
7 Immunodeficiency with normal or hyperimmunoglobulinemia <sup>44</sup>	+	+	
8 Immunodeficiency with ataxia telangiectasia <sup>83,84,86,87,90,92,138</sup>	+	+	
9 Immunodeficiency with thrombocytopenia and eczema (Wiskott Aldrich) <sup>78,99,101,102,103,110</sup>	+	+	
10 Immunodeficiency with short limbed dwarfism <sup>119,117</sup>	+	+	
11 Immunodeficiency with generalized hematopoietic hypoplasia (reticular dysgenesis) <sup>118,123,134</sup>	+	+	+
12 Severe combined immunodeficiency			
(a) Autosomal recessive <sup>125,119,140,142,151,160</sup>	+	+	+
(b) Sex linked <sup>113,119,142,151,160</sup>	+	+	+
(c) Sporadic <sup>140</sup>	+	+	+
13 Variable immunodeficiency (common largely unclassified) <sup>4,10,21,122,141,154,155,157,159,167</sup>	+	+	(sometimes)

<sup>a</sup>See also Figure 7-5 (Page 294)

World Health Organization will be used throughout this chapter, but eponyms will be given where this facilitates understanding or access to the older literature.

A comprehensive classification of acquired immune deficiency syndromes is presented in Table 44-2. Only those of special hematologic interest are dealt with in detail here, but appropriate references are listed for all. The immunosuppressive agents are discussed in the chapter on chemotherapy of leukemia and lymphomas (Chapter 55).

### Infantile Sex-Linked Agammaglobulinemia (Bruton's Agammaglobulinemia)<sup>4,5,10,12,22</sup>

#### Clinical Manifestations

Infantile sex-linked agammaglobulinemia was the first immunologic deficiency disease

to be described in detail.<sup>5</sup> It is characterized by an almost complete lack of B-cell function; once the transplacentally acquired maternal gamma globulin is lost, serum gamma globulins of all classes reach very low levels and in many individuals concentrations of 15 mg/dl or less are found. All of these patients produce antibodies poorly and many cannot produce any detectable levels of specific antibodies, even after repeated and intensive antigenic stimulation. As a result, boys with sex-linked agammaglobulinemia suffer from recurring pyogenic infections that usually involve the lungs, sinuses, inner ears, meninges, or intestinal tract. Highly encapsulated organisms are the most common offenders and among these pneumococci are preeminent, followed by *Hemophilus influenzae*, streptococci, meningococci, staphylococci, and *Pseudomonas aeruginosa*.<sup>10,12</sup> In contrast to their extreme susceptibility to pyogenic infections, patients with sex-linked agammaglobulinemia experience little difficulty in

Table 44-2. Acquired Immunologic Deficiencies

- 1 "Acquired primary" hypogammaglobulinemia<sup>122,124,136,146,154</sup>  
(see under variable immunodeficiency, page 1391)
- 2 Immunodeficiency with thymoma<sup>179,183,189</sup>
- 3 External loss of immunoglobulins and lymphocytes
  - (a) Intestinal lymphangiectasia<sup>191,196</sup> 211 216 217,218
  - (b) Other forms of protein losing enteropathy<sup>23</sup> 217
  - (c) Nephrotic syndrome<sup>23</sup>
  - (d) Exfoliative dermatitis<sup>23</sup>
- 4 Lymphoproliferative diseases and other malignant conditions
  - (a) Multiple myeloma and Waldenstrom's macroglobulinemia<sup>125,240,254,279</sup> 290 308
  - (b) Hodgkin's disease<sup>12</sup> 221 225 234 237,270 273 311,312
  - (c) Other lymphomas
    - (1) Lymphosarcoma<sup>229</sup> 249,254,273 280 289 294
    - (2) Reticulum cell sarcoma<sup>278</sup> 257 280 299
    - (3) Kaposi's sarcoma<sup>240b,278</sup>
    - (4) Burkitt's lymphoma<sup>239</sup> 284 310,318 319
  - (d) Chronic lymphocytic leukemia<sup>232</sup> 240,246,267,281,288 301
  - (e) Acute leukemia<sup>241</sup> 256 258 261 272 274
  - (f) Non-lymphoid malignancies<sup>238</sup> 242 244,263,268 285,315
- 5 Sarcoidosis<sup>237</sup> 265 266 275
- 6 Immunologic defects associated with chronic low grade infections
  - (a) Chronic mucocutaneous candidiasis<sup>170,182</sup> 186,187,197 201,205,213
  - (b) Lepromatous leprosy<sup>195</sup> 202 204 213 214 219
  - (c) Disseminated cutaneous leishmaniasis<sup>213</sup>
  - (d) Other chronic mycoses<sup>213</sup>
- 7 Viral infections<sup>171</sup> 171 193 203 207 208,209 283 287
- 8 Immunosuppressive therapy
  - (a) Drugs<sup>167</sup> 174 175 177 188 190 192,196,203,212
  - (b) Antilymphocyte serum<sup>169</sup> 200 304
- 9 Miscellaneous
  - (a) Uremia<sup>220</sup>
  - (b) Malnutrition<sup>172,176,210</sup>
  - (c) Iron deficiency<sup>134</sup>
  - (d) Senescence<sup>181,199,314a</sup>

combating recurring viral or fungal infections and react appropriately to smallpox vaccination.<sup>7,18</sup> The common viral diseases of childhood such as measles, German measles, mumps, and chickenpox are usually weathered in normal fashion. One exception to this general pattern of behavior appears to be an increased susceptibility of these patients to the infectious hepatitis virus; infection with this virus may lead to a progressively active or fulminating form of hepatitis resulting in death.<sup>20</sup> This susceptibility must be borne in mind when transfusion therapy is planned for patients with agammaglobulinemia. A number of patients have died from infection with *Pneumocystis carinii*, but successful ther-

apy with pentamidine isethionate has been reported.<sup>23</sup>

In spite of their inability to produce antibodies against most ordinary antigens, patients with agammaglobulinemia are not free from illnesses usually considered to be immunologic in nature. Indeed, diseases such as chronic synovitis, rheumatoid arthritis, dermatomyositis, scleroderma, and diffuse vasculitis occur with inordinate frequency, perhaps approximating an incidence some 20 to 30 times greater than that in the general population.<sup>11,13,19</sup> The rheumatoid arthritis, however, is usually, though not always, rheumatoid-factor negative.<sup>27</sup> In addition, hemolytic anemia, sometimes Coombs' positive,

and episodes of leukopenia and thrombocytopenia have been described.<sup>13</sup> A number of patients have developed amyloidosis. Steatorrhea is not uncommon in hypogammaglobulinemic patients. Its incidence is particularly high in those with the idiopathic acquired forms (see below), but several cases also have been reported in patients with the congenital varieties.<sup>3,4,16</sup> Patients with agammaglobulinemia also develop leukemia and lymphoid malignant diseases with exceptional frequency<sup>13,21</sup>; the incidence has been estimated at about 5%.<sup>9</sup> The high incidence of leukemia in this condition is of interest in view of the demonstrated importance of humoral mechanisms in the defense against certain forms of mouse leukemia.<sup>14</sup>

On physical examination, patients with sex-linked agammaglobulinemia show evidence of chronic sepsis and its complications. In untreated patients, severe growth retardation may be noted. Lymphoid tissues such as tonsils and nodes accessible to palpation are grossly normal.

### Immunologic Findings

1. The lymphocyte count is normal, but most of the lymphocytes are T-cells; B lymphocytes bearing all classes of surface immunoglobulins are lacking in the blood and bone marrow of most patients,<sup>1a,2,8,15</sup> while some have low concentrations of IgM- and IgA-bearing cells but no IgG-bearing B cells.<sup>8</sup> Plasma cells are greatly reduced or absent from the marrow,<sup>1</sup> the lymph nodes, and the lamina propria of the glands and bowels.

2. All classes of immunoglobulins, including the secretory immunoglobulins, are deficient. Isohemagglutinin titers and heterophil titers are very low or zero and antibody responses, even after repeated antigenic stimulation, are extremely deficient or completely absent. Reaction to the Schick test remains positive even after repeated immunizations with diphtheria toxoid, although it may become negative if patients are treated with exogenous gamma globulin containing diphtheria antitoxin.

3. Abnormalities of complement have

been described.<sup>24</sup> The concentration of the C1q subunit of C1 is often one half to two thirds of the normal mean, but the C1s activity is normal (see Chapter 7 for a discussion of complement). C1 hemolytic activity is variably depressed.<sup>24</sup>

4. Cellular immune responses are intact; delayed hypersensitivity responses to such antigens as *Candida*, diphtheria toxoid, mumps, and tuberculin are normal and deliberate sensitization to contact allergens such as 2,4-dinitrochlorobenzene (DNCB) usually is successful. In vitro lymphocyte proliferation in response to phytohemagglutinin (PHA), allogeneic cells, and appropriate antigens to which the patient has been previously sensitized is normal. Allogeneic skin grafts are rejected normally, although perhaps a few days later than in completely immunocompetent individuals.<sup>13</sup>

### Pathology

The basic structure of the thymus is intact although a certain amount of stress involution often is seen. The paracortical regions of nodes are usually normal, but the follicles are poorly developed and lack germinal centers even in specifically stimulated nodes. Plasma cells are lacking. The tonsils have poorly developed crypts and lack germinal centers and plasma cells; small accumulations of lymphocytes may be found in the pharyngeal areas. The Peyer's patches are poorly developed and lack follicles, and plasma cells are absent from the lamina propria of the intestinal tract and the secretory glands.

### Treatment

The mainstay of therapy is the monthly injection of alcohol- or ether-fractionated immunoglobulin at a minimum dose of 100 to 200 mg/kg of body weight/month. Injections of 100 mg/kg will result in an increase of serum IgG of 100 to 200 mg/dl. It is probably wise to aim for minimum residual serum levels of at least 200 mg/dl just prior to the next injection. This level is usually not difficult to achieve because of the marked decrease in the fractional catabolic rate of



immunoglobulins in hypogammaglobulinemic patients if there is no gastrointestinal protein loss.<sup>28</sup> With regular injections of gamma globulin, patients with sex-linked hypogammaglobulinemia have reached adult life; they rarely suffer from specific viral exanthems and a statistically significant beneficial effect on the incidence of bacterial infections was shown in a controlled trial of patients with hypogammaglobulinemia.<sup>17</sup>

Most available gamma globulin preparations consist largely of IgG (95%) with about 5% IgA. The effect of treatment is thus mainly confined to antibodies within the circulation and the interstitial fluids. It is therefore unlikely that gamma globulin injections serve the functions of secretory IgA; indeed it has been shown that, while patients can be relieved of their systemic infections such as pneumonia, meningitis, and septicemia, they have continued difficulty in combating infections of the mucous surfaces, particularly those of the respiratory tract, unless they are capable of producing at least small amounts of secretory IgA.<sup>25</sup>

Injections of gamma globulin may be associated with a variety of reactions including local pain, fever, rashes, dyspnea, hypotension, collapse, and even death, but these are rare in sex-linked agammaglobulinemia. The causes of these reactions are unclear; some have been thought to be complement mediated, whereas others have been attributed to antibodies against IgG or gamma globulin allotypes.<sup>6</sup> Some reactions are clearly associated with aggregation of gamma globulins within injected preparations; various tests for aggregation are available<sup>6</sup> and should be used whenever such reactions are suspected. Preparations intended for intramuscular use must never be given intravenously because of the frequency of severe reactions.

Hyperimmune immunoglobulin from normal donors who have recently recovered from specific infections may be of value for agammaglobulinemic patients exposed to these infections.

The intravenous infusion of plasma from selected donors permits replacement of a wider range of immunoglobulins.<sup>6a,26</sup> In addition, larger quantities of gamma globulins

can be given and this permits treatments to be more widely spaced. Such therapy is of special benefit in patients with severe hypogammaglobulinemia, but extreme care must be taken in the selection of donors because of the poor prognosis of agammaglobulinemic patients with infectious hepatitis.<sup>20</sup> Plasma should not be given to individuals with anti-IgA antibodies unless the plasma is derived from IgA-deficient donors.

Since recurrent sinobronchial infections are little affected by gamma globulin injections, prophylaxis with continuing postural drainage, deep-breathing exercises, and other means of maintaining good pulmonary hygiene are mandatory features of long-term management. Treatment to combat individual infections should be given early, employing full doses of specific antibiotics, preferably of limited range. In addition, short courses of antibiotics to eliminate offending organisms are sometimes indicated even in the absence of clinical signs of infections, but prolonged antibiotic prophylaxis is unwise in most instances.

## Selective Immunoglobulin Deficiency (Especially IgA)<sup>16,35,37,38,40,42,43,45,47,50</sup>

### Clinical Manifestations

A marked deficiency of IgA in serum and secretions is not uncommonly found<sup>37,39,42,43,47</sup>; its incidence may be in excess of 1:700 in unselected clinically healthy individuals or blood donors.<sup>37,43</sup> In addition, however, a large number of individuals have been described who have had significant disease in association with selective IgA deficiency.<sup>16,33,38,40,50</sup> In such patients the clinical manifestations are of three major types.<sup>16,33,41</sup>

*Sinopulmonary disease* is seen in about one to two thirds of these patients<sup>33,46</sup> and usually takes the form of recurrent upper respiratory tract infection. In patients with selective IgA deficiency, respiratory disease usually is mild or only moderately severe<sup>16,35</sup> in contrast to

the severe manifestations seen in patients lacking all classes of immunoglobulins. It is of interest, however, that the incidence of respiratory tract infections is lower in patients who lack both IgE and IgA (but not IgG and IgM) than in those lacking IgA only.<sup>46</sup> The reasons for this difference are not understood. Severe asthma also occurs with increased frequency in patients with selective IgA deficiency.<sup>35</sup> This is of interest in view of the fact that IgA is the major blocking antibody found in secretions following hyposensitization.<sup>49</sup> An apparent association between pulmonary hemosiderosis and selective IgA deficiency also has been recognized.<sup>13</sup>

A *sprue-like syndrome* occurs predominantly in adults with IgA deficiency. Its clinical manifestations are surprisingly similar to those of common, nontropical sprue, including improvement on a gluten-free diet,<sup>16</sup> although IgA levels do not improve concomitantly. Some authors have been impressed by the lack of an associated susceptibility to respiratory infections.<sup>16</sup> Histologically there is villous atrophy of the duodenal and jejunal epithelium and scarcity of IgA-producing cells. In contrast, the number of IgM-producing cells may be markedly increased.<sup>16,43</sup> Two patients were found to have anti-ileum basement membrane antibodies,<sup>35</sup> but the role of this antibody in the pathogenesis of the disease is unknown.

Gastric adenocarcinoma has been present in several patients with various types of IgA deficiency, including one individual with a selective IgA deficiency.<sup>9</sup>

A large number of "autoimmune" diseases occur in association with IgA deficiency.<sup>35</sup> Rheumatoid arthritis and systemic lupus erythematosus appear to be the most common, but dermatomyositis, pernicious anemia, thyroiditis, Addison's disease, chronic active hepatitis, and Coombs'-positive hemolytic anemia have been described. The relationship of the IgA deficiency to the development of autoimmune phenomena is not clear but it has been suggested that secretory immunoglobulins may normally serve to block viral, bacterial, and other antigens, which, if

allowed to enter, may lead to the production of autoimmune phenomena.<sup>41</sup>

### Inheritance

Although the majority of cases occur sporadically, in some families genetic patterns which suggest autosomal recessive or autosomal dominant inheritance have been established.<sup>35,45</sup> In addition, IgA deficiency appears to be particularly common in relatives of patients with various other types of congenital agammaglobulinemia.<sup>16</sup> Selective IgA deficiency occurs frequently in association with abnormalities of chromosome 18.<sup>35</sup> In addition, some children have IgA deficiency together with a variable mixture of congenital malformations, mental retardation, and epilepsy.<sup>35,50</sup>

Occasional well-documented cases of acquired selective IgA deficiency have been reported.<sup>35</sup>

### Immunologic Findings

1. The lymphocyte count is within the normal range. Normal numbers of plasma cells are found in the bone marrow and lymphoid organs, but among these cells the number producing IgA is greatly reduced or absent, particularly in the lamina propria. Surprisingly, however, the number of circulating IgA-bearing lymphocytes is either normal or increased,<sup>51</sup> suggesting a defect in differentiation from lymphocytes to plasma cells. In the gut there is often a compensatory increase in IgM-producing cells.<sup>48</sup>

2. Both exocrine and circulatory IgA's are absent, but other immunoglobulin levels generally are normal. IgM levels in secretions may be increased in some patients.<sup>48</sup> Antibody responses are normal with the exception of IgA antibodies.<sup>44</sup>

3. Cellular immune responses are normal.

4. A number of abnormal antibodies and immunoglobulins have been described, including: (a) A high incidence of antimilk and antiovine antibodies.<sup>35,45</sup> The latter are IgG antibodies directed against normal serum from cows, goats, and sheep that also show cross reactivity with milk<sup>35</sup>; these antibodies

appear to be directed against the IgM of the animal concerned. (b) Antibodies against human IgA are found in about 40% of patients and a similar number have anti-IgG antibodies.<sup>35</sup> There appears to be no correlation with previous gamma globulin therapy or plasma administrations. (c) Low molecular-weight IgM (7S IgM) is noted in some patients. (d) A variety of autoantibodies have been described including antinuclear antibodies, antithyroid antibodies, antithyroglobulin antibodies, anti-smooth muscle antibodies, and anti-bile canaliculi antibodies.<sup>35</sup> In many patients these antibodies are not associated with overt autoimmune disease. The antibodies reacting with ileal basement membrane have been described previously (page 1382). Nodular lymphoid hyperplasia within the lamina propria has been reported in patients with other forms of agammaglobulinemic lymphoid enteropathy, but is not found in patients with isolated IgA deficient sprue.<sup>16</sup>

### Treatment

The general care of patients with sinopulmonary complications is similar to that described for agammaglobulinemic patients (page 1381), but injections of gamma globulin are seldom of any use. When patients with selective IgA deficiency develop anti-IgA antibodies,<sup>35</sup> severe hypersensitive reactions and even lethal anaphylaxis may follow the transfusion of normal plasma or blood. Such individuals must be transfused only with blood derived from IgA-deficient donors.

Patients with selective IgE deficiency also have been reported.<sup>46</sup> Most are free from recurrent infections.

## Transient Hypogammaglobulinemia of Infancy<sup>23,61,62</sup>

### Clinical Manifestations

At birth the full-term human infant is amply endowed with passively acquired maternal gamma globulin (1000 mg/dl) which

consists mostly of IgG (see Chapter 7). Because the half-life of these antibodies is approximately 21 days the newborn's gamma globulin levels drop rapidly. Ordinarily, however, the normal newborn begins to synthesize IgM antibodies at birth and attains 75% of adult levels by one year of age. Appreciable IgG synthesis starts a little later, probably during the second month of life and levels of about 800 mg/dl are reached by the end of the first year. As a result of declining maternal gamma globulin levels and increasing gamma globulin synthesis by the infant, the nadir of the gamma globulin levels is ordinarily reached between the third and fifth month of life. Rarely there is an abnormally prolonged delay in the onset of gamma globulin synthesis by the infant and levels may temporarily drop to those ordinarily present in infants with sex-linked agammaglobulinemia. This unphysiologic event has been designated "transient hypogammaglobulinemia of infancy." Its incidence is the same in males and females. Recovery usually occurs when the infant is between 9 and 15 months of age, but some individuals may have persistently low IgG levels later in life as well.<sup>63</sup> Before normal immunoglobulin synthesis begins, infants with transient hypogammaglobulinemia may display an undue susceptibility to infections of the skin, upper respiratory tract, lungs, and meninges, usually with highly encapsulated organisms of the type encountered in persons with congenital sex-linked agammaglobulinemia. At this point the two conditions may be clinically indistinguishable.

### Laboratory Findings

1. The number of circulating *lymphocytes* is normal and plasma cells, ordinarily sparse this early in life, are absent.
2. IgG is primarily depressed, but other *immunoglobulin* values may also be low. Antibody responses to most antigens are low or absent.
3. *Cellular immunity* is normal.
4. Germinal centers are lacking within the peripheral lymphoid tissues.

## Therapy

When therapy is necessary, it is similar to that described for congenital sex-linked agammaglobulinemia.

## Immunodeficiency with Hyper IgM<sup>64</sup>

Immunodeficiency with hyper IgM is a partial immunoglobulin defect characterized by deficiency of IgG and IgA, but increased levels of IgM. The disorder may be congenital or acquired and may be found in either sex but occurs most frequently as an apparent sex-linked gene defect.

Patients affected with this disease suffer from repeated bouts of infection with pyogenic organisms, even though antibody activity against some, but not all, antigens has been demonstrated within the IgM fraction. In addition, these patients frequently suffer from hemolytic anemia, recurrent or persistent neutropenia, and thrombocytopenia. Apparently they have a normal total number of B lymphocytes.

Regular prophylactic administration of IgG will prevent most of the infections.

## Thymic Hypoplasia (Pharyngeal Pouch Syndrome, Di George Syndrome)<sup>68,69,74,75,76,78</sup>

### Clinical Manifestations

The prototype of T-cell defects is the syndrome of thymic hypoplasia, which was initially recognized as such by Di George<sup>68</sup> although the first case was probably recorded as early as 1829.<sup>71</sup> This syndrome is due to faulty development of structures derived from the third and fourth pharyngeal pouches, including the thymus, the parathyroid glands, and, sometimes, the blood vessels of the neck and the esophagus. Since the lack of parathyroids leads to tetany in the neonatal period, hypocalcemia is the first clue

to the diagnosis of this disorder. Because the lack of a thymic anlage leads to deficient T-cell function (Chapter 7), these patients soon develop infections that are normally controlled by cellular immune phenomena. Thus they are particularly susceptible to infection with fungi, especially *Candida*; viruses such as vaccinia, rubeola, and cytomegalovirus; *M. tuberculosis*; atypical acid-fast bacilli; BCG; *Listeria*; and *Pneumocystis carinii*. In the absence of attempts at definitive therapy (see below), death due to disseminated sepsis usually occurs during the first year of life. Failure of growth and "runting" with virtual disappearance of all subcutaneous fat is a characteristic finding during the later stages of life.

### Laboratory Findings

1. *Lymphocyte counts* are variable, but surprisingly the number of these cells is usually within the normal range. The lymphocytes grow poorly in culture, however, and attempts to stimulate their proliferation with specific antigens, PHA, or antilymphocyte sera generally are unsuccessful.<sup>69</sup> Normal or increased numbers of plasma cells are found in the bone marrow and lymph nodes, and in the lamina propria lining hollow organs.

2. *Immunoglobulins* are found in normal concentrations, except that sometimes IgE levels may be elevated.<sup>69</sup> Antibody responses were studied in one patient and were normal for some antigens such as A and B blood group substances and *E. coli*, but were poor or completely lacking for other antigens such as diphtheria-pertussis and tetanus (DPT), poliomyelitis and inactivated measles vaccines, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida*.<sup>69</sup> Presumably the latter are thymus-dependent antigens (page 316).

3. *Cellular immunity* is almost totally lacking; there are no delayed hypersensitivity responses to antigens of known exposure, including *Candida*, and it is impossible to induce sensitization with DNCB. Allogeneic skin grafts are not rejected. The behavior of lymphocytes in vitro was discussed above.

## Pathology

Serial sections of the anterior neck and mediastinal tissues fail to reveal any thymic or parathyroid tissue. Lymph node biopsy specimens initially show depletion of thymus-dependent areas and preservation of thymus-independent areas such as follicles, germinal centers, and medullary cords. Plasma cells are abundant. At postmortem examination, marked general lymphoid depletion is seen, probably because of severe terminal sepsis.

## Treatment

Effective therapy has been accomplished by the transplantation of fetal thymic tissue.<sup>66,67</sup> This presumably provides a micro-environment that allows lymphoid precursors to differentiate into thymus-derived small lymphocytes. Unfortunately, the acquisition of immune competence will almost invariably lead to rejection of the thymic transplant and repeated thymic transplants may become necessary.<sup>41</sup> One patient, however, was well 5½ years after receiving a thymic transplant.<sup>48</sup> Apparently spontaneous recovery has also been reported.<sup>44</sup>

Since patients with thymic hypoplasia are incapable of rejecting histoincompatible cells, transfusions with whole blood containing viable immunocompetent lymphocytes may initiate rapidly fatal graft-versus-host disease. It is therefore imperative to irradiate donor blood with at least 3000 rads prior to transfusion.<sup>42</sup>

## Other Forms of Thymic Hypoplasia

A number of patients have been described who likewise manifest cell-mediated immune deficiency syndromes in the face of normal immunoglobulin levels, but have normal parathyroid function.<sup>65,70,77</sup> At postmortem examination the thymus has been absent or aplastic while plasma cells have been found in abundance. With the exception of endocrine abnormalities the clinical course of these patients has been similar to that of

patients with the pharyngeal pouch syndrome. One patient has been described, however, in whom thymic hypoplasia and defective cellular immunity were accompanied by hypothyroidism and normal parathyroid function.<sup>73</sup> It was thought that this patient suffered from a developmental defect involving the second and third pharyngeal pouches.

## Immunodeficiency with Ataxia Telangiectasia<sup>83,84,86,87,90,92</sup>

### Clinical Manifestations

Ataxia telangiectasia is a familial disease the cardinal features of which consist of (1) progressive cerebellar ataxia, (2) progressive oculocutaneous telangiectasis, (3) severe sinopulmonary infections, and (4) an extremely high incidence of lymphoreticular malignant disease. The immunologic defect is thought to involve both T-cells and B-cells but not stem cells.

The *neurologic* manifestations<sup>83,84</sup> are usually the first to appear, frequently when the child begins to walk. In addition to progressive cerebellar signs, other features include choreo-athetoid and tic-like movements, occasional myoclonic jerks, and apraxia of eye movements simulating ophthalmoplegia. Although mental deficiency is not found during the early stages of the disease, the intelligence quotient is said to drop below the normal range as the illness progresses. In the cerebellum there is severe degeneration of the Purkinje cells. Less pronounced degenerative changes are found elsewhere in the brain. Their cause is not known; it is not obviously vascular.

*Oculocutaneous telangiectases*<sup>91</sup> usually appear when the patient is five or six years of age; the bulbar conjunctiva is most characteristically involved. Cutaneous telangiectases occur mainly in the exposed areas including the ears, the bridge of the nose, and the butterfly area of the face. Eventually other sites including the neck, hands, feet, and the antecubital and popliteal areas may become

involved. In some patients, mask-like facies and sclerodermatous changes develop. The telangiectatic vessels are derived from the subpapillary venous plexuses of the skin and the conjunctival connecting venules. Other cutaneous manifestations include pigmentary disturbances such as partial albinism, malignant lesions, and infections.

*Sinopulmonary infections*<sup>82,83,88</sup> are common but not invariable components of the syndrome. Children with this complication usually suffer from progressive bronchiectasis and eventually die of resultant respiratory insufficiency and sepsis. The infections usually are due to common bacterial pathogens, but do not respond well to antibiotic therapy. The increased susceptibility to infections is due to a variably associated immune deficiency that may involve defects in cellular immunity, as well as low levels of secretory immunoglobulins in secretions and in the blood.<sup>82,83,89,90,93</sup> Approximately 60% of all the patients eventually develop IgA deficiency, but according to some investigators<sup>82</sup> the associated lack of IgE, rather than the absence of IgA alone, correlates best with increased susceptibility to sinopulmonary disease. Others have found no such correlation.<sup>86</sup>

Cancer occurs with inordinate frequency in children with ataxia telangiectasia; in at least 10%, death is due to malignant lesions of various types.<sup>9</sup> Tumors of lymphoreticular origin are most common and include reticulum cell sarcomas and other lymphomas and leukemia, but tumors such as gliomas, medulloblastomas, ovarian tumors, and gastric adenocarcinomas also have been described.<sup>9</sup> Probably the most provocative characteristic of this group of malignant diseases is the fact that in six families more than one sibling with ataxia telangiectasia developed the same type of cancer.<sup>9</sup> Gonadal dysgenesis almost bordering on agenesis is also a common associated defect.<sup>88</sup>

### Inheritance

Ataxia telangiectasia is inherited as an autosomal recessive characteristic.

### Immunologic Findings

1. Lymphocyte counts are variable, and in perhaps a third of all these patients the counts are below  $1.0 \times 10^9/l$ . Plasma cells usually are present, but their numbers may be greatly reduced on occasion.

2. IgG levels are low in one third and serum and secretory IgA levels are low in about three quarters of all the patients. Whereas IgE levels also are very low in some,<sup>46</sup> IgM levels are elevated in about three quarters of all patients. Low IgM levels are extremely rare. Antibody responses generally are poor, especially in patients with IgG deficiency.

3. Cellular immune responses are grossly abnormal in most subjects<sup>88</sup>; about three quarters of these patients show no delayed hypersensitivity to naturally acquired infections such as candidiasis and cannot be sensitized to contact vesicants such as DNCB. In addition, attempts to transfer delayed hypersensitivity reactions passively from a normally responding individual generally have failed. In one study, none of the patients could reject skin homografts normally. Since patients with ataxia telangiectasia have been known to recover normally from various types of viral infections, their cellular immune responses must have been intact for a time at least.<sup>41</sup> In vitro responses of lymphocytes to PHA, allogeneic cells, and previously encountered antigens are markedly depressed.

### Pathology

At postmortem examination the thymus is almost invariably found to be small and devoid of lymphocytes; most cells appear to be of an epithelial stromal type and Hassall's corpuscles are consistently absent.<sup>88</sup> Lymph node structure has ranged from normal to almost total depletion of thymus-dependent areas and, less frequently, of follicles.<sup>88</sup>

### Prognosis and Treatment

Prognosis in this disease is poor. The ataxia and telangiectasia become progressively

worse and by the time these patients reach their mid to late teens, most of them are confined to a wheelchair. Those with recurrent sinopulmonary sepsis usually develop bronchiectasis and eventually die of respiratory insufficiency and pneumonia. A few patients survive into the third decade of life, usually with severe neurologic disability but with few respiratory complications. Many die with lymphoreticular malignant disease.

Treatment generally is inadequate. In addition to general measures described for patients with sex-linked agammaglobulinemia (page 1381), plasma infusions are used by some physicians.<sup>81</sup>

## Immunodeficiency with Thrombocytopenia and Eczema (Wiskott-Aldrich Syndrome)<sup>98,99,101,102,103,110</sup>

### Clinical Manifestations

The Wiskott-Aldrich syndrome is characterized by a triad of clinical findings: (1) recurrent infections with a variety of organisms, due to selective deficiencies of cellular and humoral immunity; (2) moderate to severe chronic thrombocytopenia; and (3) eczema. More than 100 patients with this deficiency have been described.

The primary defect appears to consist of an inability to respond immunologically to polysaccharide and lipopolysaccharide antigens (see below). Affected individuals therefore suffer from severe gram-positive and gram-negative bacterial sepsis which may involve every organ system of the body. Defects in cellular immunity lead to infections with viruses, fungi, and *Pneumocystis carinii*. Viral infections may be overwhelming and are often due to the herpes simplex virus and other viruses ordinarily associated with a benign clinical course.

Thrombocytopenia is found in all patients and may be severe. Bleeding manifestations are common and frequently are aggravated by sepsis and the use of drugs that interfere with platelet function (Chapter 35).

The eczema has the characteristics of atopic dermatitis of infancy and can be favorably influenced by topical or systemic steroids. However, such therapy is dangerous and generally unwarranted, since it undoubtedly increases susceptibility to infection. These patients also develop other allergic manifestations with unusual frequency; many are asthmatic, some develop skin-sensitizing antibodies to common allergens, and others have had food allergies.<sup>102</sup> IgE levels are very much above normal in many of these patients.<sup>46</sup> Unusually severe local and systemic reactions to the injection of sterile bacterial vaccines also have been observed. Some patients develop transient episodes of recurrent arthritis, usually involving several joints.<sup>101</sup> The cause of this process is unknown.

Patients who do not succumb to infections or bleeding due to thrombocytopenia frequently develop various forms of lymphoreticular malignant lesions,<sup>9,111</sup> including malignant reticuloendotheliomas, reticulum cell sarcomas, and other forms of lymphomas. Occasionally tumors of nonlymphoreticular origin also occur. It is estimated that death is due to malignant disease in 10% of patients with the Wiskott-Aldrich syndrome. With better general pediatric care and consequent longer survival the incidence may rise.

In addition to the physical findings discussed above, marked "benign" lymphadenopathy is a striking feature of this disease. It may be aggravated by the systemic administration of gamma globulins.<sup>9</sup>

### Laboratory Findings

1. Lymphocyte counts are normal initially, but lymphopenia is characteristically present late in the disease. This is predominantly due to a selective loss of small, thymus-dependent (or T-) lymphocytes. Plasma cells are readily found.

2. IgG and IgA levels often are increased,<sup>100,101</sup> and normal levels of IgA and its "transport piece" (Chapter 7) are found in secretions. Serum IgE levels are markedly elevated,<sup>112</sup> but IgM levels regularly are low.<sup>100,101</sup> Hypercatabolism of IgM as well

as of the other immunoglobulins and albumin has been demonstrated,<sup>100</sup> but while increased synthetic rates more than compensate for the increased catabolism of IgG and IgA, this is not the case with IgM. In addition, antibody production to polysaccharide antigens such as blood group substances, Forssman antigens, Vi antigen, and pneumococcal polysaccharides is characteristically poor or absent and antibodies to such antigens are often predominantly of the IgM type. Antibody production to protein antigens often is normal.

3. The thymus-dependent system develops normally for a time, but defects in cellular immunity soon become apparent. Thus, when first tested, most individuals with the Wiskott-Aldrich syndrome have constant defects in cellular immunity. Delayed hypersensitivity reactions to *Candida*, mumps, streptokinase-streptodornase, and similar antigens are negative and primary sensitization to contact allergens such as DNCB is difficult or impossible. Allogeneic skin grafts survive long or indefinitely. In vitro response of lymphocytes to PHA is clearly normal, but the response to specific antigens is poor.<sup>107,108</sup>

4. Neutrophils respond well to chemotactic stimuli, and phagocytose, degranulate, and kill microorganisms normally. The reticuloendothelial system clears particulate matter in normal or accelerated fashion. The complement system is normal.

5. Thrombocytopenia is found in all patients and may range from under 10 in severely affected individuals to 50 to  $60 \times 10^9/l$  in others. Platelets show some degree of anisocytosis with a few large forms but most are of a smaller than normal size. Megakaryocytes are numerous in bone marrow preparations. Platelets from normal individuals survive normally in patients with the Wiskott-Aldrich syndrome, but the half-life of autologous platelets is drastically reduced.<sup>97</sup> In addition, it was found that platelets from patients with the Wiskott-Aldrich syndrome fail to aggregate with epinephrine and no increase in citric acid cycle activity took place on addition of polystyrene-latex particles<sup>104</sup>; in this study these reactions were also found to be depressed in the mother of

the affected siblings.<sup>104</sup> Thus thrombocytopenia in the Wiskott-Aldrich syndrome may, at least in part, be due to an "intracorpusecular" defect. Similar defects have not yet been convincingly demonstrated in the skin or lymphocytes of affected individuals.

## Pathology

The thymus is structurally normal but may show some degree of involution due to stress. Germinal centers and plasma cells are abundant in the spleen and lymph nodes. With time, there is progressive depletion of small lymphocytes from the paracortical (thymus-dependent) regions. Reticulum cell hyperplasia frequently becomes evident in lymphoreticular tissues and the amount seen may correlate roughly with the age of the patient and the degree to which the patient has suffered from infections.<sup>101</sup>

## Prognosis and Treatment

Most boys with the Wiskott-Aldrich syndrome die during early childhood. The mean age at death in one study was  $3\frac{1}{2}$  years<sup>101</sup>; very few patients survive past puberty. Patients usually die of infection, hemorrhage, or lymphoreticular malignant disease, the percentage due to each cause decreasing in the order listed.

Until recently, therapy consisted of antibiotic administration, pulmonary hygiene, platelet transfusions, and plasma infusions. Because blood transfusions carry the risk of severe graft-versus-host disease once defects of cellular immunity have developed, it is imperative that all blood products be irradiated with 3000 rads before infusion. More recently, bone marrow transplantation has been tried,<sup>96,105,106</sup> but its effect does not seem to be as clearly helpful as that of transplants in patients with lymphopenic immunologic deficiency (see page 1390). Nevertheless, it may decrease the requirements for platelet transfusions and the frequency of infections. Similar changes in the immune status may be achieved in some patients by the repeated injection of leukocyte dialysates from normal donors,<sup>6a,103,109</sup> presumably because these



preparations contain "transfer factor" (see Chapter 7). In responding patients there is a decreased incidence of infections, and the number of circulating T-cells is increased.<sup>6a, 113</sup> In addition, platelet levels may rise and the eczema may disappear.

## Severe Combined Immunodeficiency (SCI)

(a) Autosomal Recessive (Swiss Agammaglobulinemia)<sup>135, 139, 140, 142, 151, 160</sup>

(b) Sex-Linked (Thymic Alymphoplasia)<sup>133, 139, 142, 158, 160</sup>

(c) ?Sporadic<sup>140</sup>

### Clinical Manifestations

The term "severe combined immunodeficiency" (SCI) describes a condition characterized by an almost total lack of antibody production and cellular immune function.<sup>128</sup> Autosomal recessive and sex-linked inheritance patterns are recognized. These had previously been designated "Swiss agammaglobulinemia" and "thymic alymphoplasia," respectively. The clinical manifestations of both of these conditions are similar although patients with the sex-linked form of the disease tend to be less severely depleted of lymphocytes and probably live longer than do patients with the autosomal recessively inherited variety.<sup>140</sup> A sporadically occurring form of the disease has been postulated,<sup>140</sup> but available pedigree analyses are insufficient to establish nongenetic mechanisms. The basic immunologic defect in severe combined immunodeficiency probably is at the level of the lymphoid stem cell and is expressed as absent B- and T-cell function.

Babies with SCI usually appear healthy at birth and start to gain weight normally. However, within the first few weeks of life they begin to develop infections of the skin, the bronchopulmonary tree, and the gastrointestinal tract and from then on infections remain with these patients for the rest of their brief lives. As a result, the clinical course is characterized by repeated bouts of pneumonia, otitis media, pyoderma, meningitis, gas-

troenteritis, and septicemia due to low-grade pathogens or more aggressive bacteria. Candida infection of the respiratory and intestinal tracts develops in almost all patients and frequently leads to systemic dissemination. In addition, affected babies are highly susceptible to viral infections of all kinds and death may follow exposure to varicella, rubeola, or vaccinia. A fulminating, rapidly fatal form of hepatitis similar to that in patients with infantile sex-linked agammaglobulinemia (page 1379) makes plasma infusions from poorly screened donors especially dangerous.<sup>41</sup> Death may also result from disseminated fungal infections, BCG vaccinations,<sup>120</sup> or *Pneumocystis carinii* infections. Thus patients with severe combined immunodeficiency are highly susceptible to all forms of pathogens.

With the onset of infections the infant fails to thrive and soon becomes extremely thin and emaciated with lax folds of skin enveloping a bony frame. The wasting syndrome is akin to that seen in neonatally thymectomized mice and could probably be prevented by raising the baby in a germ-free environment. Skin rashes develop frequently and may resemble morbilliform exanthema or what looks like full-blown atopic dermatitis. Sometimes the hemolytic uremic syndrome or disseminated intravascular coagulation develops terminally.

### Immunologic Findings

1. The peripheral blood lymphocyte count is invariably low in the sex-linked form and very low in the autosomal recessive form. There are very few lymphocytes in the lymphatic channels, lymphoid organs, and the bone marrow, although occasional nests of lymphocytes may be present in patients with the sex-linked variety of severe combined immunodeficiency.

2. Once maternal immunoglobulins have disappeared, all immunoglobulin classes are absent or extremely deficient. No secretory immunoglobulins are found with the exception of elevated IgE levels in occasional patients.<sup>46</sup> Antibody production is absent or extremely deficient. Severe depression of the

Clq component of complement has been observed, especially in the autosomal recessive form of the disease.<sup>24</sup>

3. Cellular immune responses are absent by all parameters; delayed hypersensitivity reactions to all antigens are negative and it is impossible to induce skin sensitivity to DNCB. Allogeneic skin grafts survive indefinitely. In vitro, lymphocytes from patients with severe combined immunodeficiency fail to proliferate in the presence of PHA or specific antigens. Since the lymphocyte response to PHA is mature at birth in normal infants, this provides a useful test for the exclusion of the possibility of severe combined immunodeficiency when this diagnosis is suspected because of family history or other reasons. Lymphocytes from patients with severe combined immunodeficiency generally do not respond in mixed leukocyte reactions, although weak responses may sometimes occur in individuals with the sex-linked form of the disease.<sup>118,130</sup> Macrophage function is normal.<sup>128</sup>

4. Other hematologic changes: The red cells are normal although eventually anemia develops because of infection or iron deficiency. Acute episodes of hemolysis have been described in at least one patient; these were due to an acute hemolytic uremic syndrome. Thrombocytopenia usually is normal, as is myelopoiesis. Occasionally pancytopenia develops terminally.

A deficiency of the red cell enzyme *adenosine deaminase* has been reported in several patients suffering from severe combined immune deficiency.<sup>124,130a,139a</sup> Parents and some relatives have lower than normal adenosine deaminase activity and may be carriers of the defect. Since the adenosine deaminase enzymes found in lymphocytes and red cells have similar properties, their activity in lymphocytes and red cells is thought to be controlled by the same gene.<sup>130a</sup> A causative relationship between abnormal lymphocyte function and adenosine deaminase deficiency has been postulated.

### Pathology

The thymus is tiny and vestigial, lacking recognizable Hassall's corpuscles and lym-

phocytes and consisting almost entirely of epithelial cells. It frequently is ectopic and may be found high up in the neck. The peripheral lymphoid tissues are severely hypoplastic; the pathologist may have difficulty in finding any lymph nodes at all. The spleen, the lymph nodes, and the gastrointestinal tract are almost totally devoid of lymphocytes and no lymphoid follicles, germinal centers, Peyer's patches, or plasma cells are found.<sup>142</sup> In some patients there is a marked proliferation of histiocytes which may mimic findings in the Letterer-Siwe syndrome.<sup>236</sup> Patients with the sex-linked form of the disease sometimes have a small amount of normally organized lymphoid tissue in the lymph nodes, spleen, and bone marrow.<sup>61</sup>

### Prognosis and Treatment

Without marrow transplantation (see below), SCI is universally fatal. The mean age at death in patients with the autosomally inherited diseases is five months; in those with the sex-linked form, ten months.<sup>140</sup> Death is due to overwhelming sepsis.

Conventional forms of treatment including antibiotic therapy, gamma globulin injections, and other supportive measures usually are of little long-term benefit. When a normal histocompatible sibling (Chapter 12) is available, bone marrow transplantation may be worthwhile.<sup>117,127,149,165</sup> One such patient received a sibling transplant at the age of six months and remained immunologically and hematologically reconstituted 2½ years later.<sup>127</sup> A successful transplant of bone marrow from an histocompatible uncle has also been reported.<sup>162</sup>

### Immunodeficiency with Short-Limbed Dwarfism<sup>129,137</sup>

Clinically and immunologically this syndrome closely resembles severe combined immunodeficiency with autosomal recessive inheritance (see above). It is, however, characterized by two additional features: lymphadenopathy and short-limbed dwarfism with ectodermal dysplasia. The nodes,

though large, are devoid of lymphocytes and are structurally similar to those characteristic of severe combined immunodeficiency. The dwarfism is distinctive for this syndrome and is not found separately or in conjunction with any other disease. Similarity has been noted between this syndrome and that occurring in children suffering from *cartilage-hair hypoplasia*,<sup>150,152</sup> although in the latter disease the immune defect appears to be mainly cellular<sup>150</sup>; gamma globulin levels are normal and patients are capable of producing antibodies to a variety of viral and bacterial antigens.<sup>150</sup>

## Variable Immunodeficiency (Common, Largely Unclassified)

4, 10, 23, 122, 141, 154, 156, 157, 159, 167

The extraordinary variability of immunologic findings makes classification of immune deficiency syndromes most difficult. The majority of immune defects cannot be unequivocally categorized; instead they are grouped under the heading "variable immunodeficiency," which includes diseases previously classified as (1) congenital non-sex-linked hypogammaglobulinemia (sporadic), (2) primary dysgammaglobulinemia of both childhood and adult life, and (3) acquired primary hypogammaglobulinemia, which may actually be a generically determined disorder.<sup>122,124,136,146,164</sup> Careful analysis in affected patients will in time undoubtedly yield several homogeneous syndromes.

The term "*dysgammaglobulinemia*" has been used to describe two different clinical situations.<sup>157</sup> It has been applied to patients who have serum immunoglobulins but who are nevertheless unable to produce detectable circulating antibody on repeated antigenic challenge (immunoparesis of Giedion and Scheidegger).<sup>131,132</sup> More commonly, however, the term is used to describe an immunodeficiency characterized by the presence of one or two immunoglobulins but lack of the others. Although several combinations are possible, the most common abnormality of this type is characterized by the absence of IgG and IgA and the presence of abnormally

high levels of IgM and, sometimes, IgD. This abnormality has previously been called dysgammaglobulinemia type I, but use of this designation is to be discouraged.<sup>41</sup> Congenital and acquired forms occur. The congenital variety has been described in boys only, whereas the acquired form is found in both males and females.<sup>157</sup>

## Clinical Manifestations

In individual patients, demonstrated immunologic deficiencies correlate well with the expected clinical findings. Thus adults or children with sporadic, non-sex-linked hypogammaglobulinemia have low levels of all immunoglobulins, and produce antibodies poorly in response to antigenic stimulation. They are plagued by recurrent infections with encapsulated pyogenic pathogens such as pneumococcus, streptococcus, *H. influenza*, *Pseudomonas*, and meningococcus and many suffer from intestinal giardiasis.<sup>2a,119,124</sup> In these patients there is an even higher incidence of other complications, such as autoimmune phenomena including pernicious anemia,<sup>2a,161</sup> malabsorption syndromes,<sup>119,143</sup> and allergy,<sup>125,126</sup> than one finds in patients suffering from congenital sex-linked agammaglobulinemia. Patients with "dysgammaglobulinemia" are particularly prone to develop indolent oral ulcers and widespread infection with verruca vulgaris; almost all suffer from cyclical neutropenia and some develop hemolytic anemia and thrombocytopenia.

Patients with late-onset hypogammaglobulinemia also are quite prone to develop lymphoid malignant disease, including chronic lymphocytic leukemia, lymphosarcoma, and malignant reticuloses, as well as nonlymphoid tumors such as carcinomas, sarcomas, and epitheliomas.<sup>9</sup> These malignant tumors often occur many years after the initial diagnosis of immunodeficiency was made.

## Laboratory Findings

1. Lymphocytes are usually present in normal numbers, but there may be a variable decrease in the number of those bearing sur-

face immunoglobulins (B-cells).<sup>12,8</sup> Occasionally, however, the number of immunoglobulin-bearing cells is increased even though hypogammaglobulinemia is present.<sup>121,155</sup> In such individuals the defect may consist of a block in the differentiation of lymphocytes to plasma cells.

2. Immunoglobulin deficits are invariably present, but the class involved and the degree of change are variable. Unusual distributions of IgG subgroups have been described,<sup>166</sup> as well as abnormal ratios of kappa and lambda chains and poor fixation of complement. Antibody production is variably deficient. Patients with low IgG and IgA but high IgM frequently have very high titers of isohemagglutinins and anti-Forssman antibodies.<sup>157</sup>

3. Deficient cellular immunity is often encountered, especially late in the disease.<sup>41 149 147 163</sup> As defects in cellular immunity become apparent, *in vivo* and *in vitro* tests of lymphoid function become abnormal.

### Pathologic Findings

The pathologic findings are variable but generally predictable on the basis of clinical findings. Occasionally, striking reticular and follicular hyperplasia that may mimic that associated with "benign lymphomas" is present. Sometimes marked hyperplasia of the lymphoid tissue of the ileum and even of the colon is noted.<sup>41</sup>

## Other Congenital Disorders of Host Defense against Infections

Defects of the complement system also are associated with recurrent infections, usually involving pyogenic bacteria. These include C3 deficiency,<sup>115 116</sup> C5 dysfunction,<sup>153</sup> and defects of the alternate pathway of C3 activation in patients with sickle cell disease.<sup>143</sup> (See Chapter 7 for details of the complement system.) Inherited defects of phagocytic function are discussed in Chapter 42.

## Acquired Immune Deficiency Syndromes

### Immunodeficiency with Thymoma<sup>179,183,189</sup>

#### Clinical Manifestations

The association of thymoma with hypogammaglobulinemia was first documented by Good in 1954 and since then a considerable number of patients with similar manifestations have been described.<sup>183</sup> The syndrome is most commonly encountered in the sixth decade of life, although the first symptoms may appear anytime from the third to the eighth decade. All patients with this type of immunodeficiency had been healthy previously. Members of both sexes are affected.

The thymic tumor may be discovered on routine chest x-ray examination, often before there is evidence of an immunologic deficiency syndrome. It may be so small as to be invisible on x-ray films or it may be huge; reported weights have ranged from 40 to over 2000 g with an average of 500 to 600 g.<sup>183</sup>

Once the immune deficiency becomes established, most patients complain of severe weakness and weight loss. Almost all suffer from recurring respiratory tract diseases including sinusitis, chronic bronchitis, and pneumonia. Many subsequently develop bronchiectasis, pulmonary fibrosis, and emphysema. The most frequently encountered organisms are pneumococci, but *H. influenza*, *klebsiella*, and other organisms are also present. Other infections with manifestations such as stomatitis, pyoderma, pyelonephritis, gastroenteritis, meningitis, and septicemia have been reported. *Candida albicans* infections become particularly bothersome later in the disease. Diarrhea occurs in at least a third of all the subjects and protein-losing enteropathy is often present. Symptoms of myasthenia gravis have been reported in some of these patients.<sup>183</sup>

Benign thymomas often occur in conjunction with specific hematologic abnormalities. Thus, according to one study, 6% of all pa-

tients with thymoma suffer from pure red cell aplasia or aplastic anemia<sup>204</sup> (Chapter 56); however, in those patients who have hypogammaglobulinemia in addition to thymoma, the incidence of hematologic abnormalities, especially hypoplastic or aplastic anemia, is much higher and may reach 30%.<sup>183</sup>

### Immunologic Findings

1. Lymphocyte counts are often low initially and decline further with time, often to extremely low levels. Plasma cells are absent from the bone marrow, the lymphoid organs, and the gut.

2. Severe hypogammaglobulinemia is present in all subjects; serum IgG levels are often barely detectable while IgA and IgM usually are completely absent in patients with fully developed cases. When serum IgA is lacking, it is likely that secretory IgA is also absent since this appears to be a consistent correlation in other immune deficiency syndromes.<sup>43</sup> Humoral antibody responses to all antigens are markedly reduced.

3. Cellular immunity has not been studied as extensively as humoral immunity. Nevertheless, progressively declining peripheral lymphocyte counts and inability to manifest delayed hypersensitivity reactions to natural antigens and contact allergens such as DNFB have been reported. In addition, prolonged survival of skin allografts and poor *in vitro* lymphocyte responses have been noted.

4. Associated hematologic abnormalities include anemia, reticulocytopenia, and, occasionally, leukopenia and eosinopenia. The marrow may contain few or no red cell precursors when red cell aplasia is also present.

### Pathology

Histologically most tumors are classified as spindle cell thymomas, presumably of epithelial origin, although occasionally epithelial, lymphoepithelial, or lymphocytic tumors are described. Plasma cells are usually absent from all areas. Germinal centers are lacking in lymph nodes and elsewhere, although the paracortical, thymus-dependent areas of

nodes may contain a considerable number of lymphocytes, especially early in the disease.<sup>183</sup>

### Treatment

No specific therapy is available. General measures, as described for patients with sex-linked agammaglobulinemia, may be of some help. Removal of the thymoma does not correct the immunologic deficiencies,<sup>41</sup> although it may be helpful in the treatment of the aplastic anemia.<sup>183</sup>

### Intestinal Lymphangiectasia<sup>198,211,216,217,218</sup>

#### Clinical Manifestations

Patients with intestinal lymphangiectasia have an anatomic defect in the intestinal lymphatics which leads to loss of serum proteins and lymphocytes into the bowel.<sup>211</sup> Two types of defects have been demonstrated: (1) relatively large lymphatic fistulas emptying into the upper small intestine,<sup>198,216</sup> and (2) dilatation of the lacteals of the intestinal villi suggestive of obstruction or stenosis of major lymphatic channels.<sup>168</sup> Lymphatic fluid has been shown to leak directly into the bowel lumen of patients with intestinal lymphangiectasia. In addition to a combined immunodeficiency syndrome, peripheral edema and ascites due to hypoalbuminemia may be present. While patients with intestinal lymphangiectasia suffer from hypogammaglobulinemia and profound anergy because of lymphocytopenia (see below), they do not often suffer from serious infections,<sup>217</sup> unlike patients with severe combined immunodeficiency (page 1389).

#### Immunologic Findings

1. Moderate to marked lymphocytopenia with mean values of  $0.7 \times 10^9/1.2^{18}$

2. Moderate to severe decrease of all major immunoglobulin classes, accompanied by increased fractional catabolic rate of intra-

vascular protein due to protein loss into the gastrointestinal tract<sup>211</sup>; immunoglobulin synthesis rates only slightly elevated. Antibodies to specific antigens (eg, Vi, tularemia) are produced in normal fashion, although the maximal titers are lower than in controls.<sup>211</sup>

3. Cellular immune responses, including delayed hypersensitivity reactions to PPD, mumps, trichophyton, and *Candida* antigens, are markedly reduced<sup>217,218</sup> and allogeneic skin grafts may survive indefinitely.<sup>217,218</sup>

4. Malabsorption may lead to anemia and clotting abnormalities due to vitamin K deficiency.

### Treatment

When a major lymphatic fistula into the small intestine can be demonstrated by lymphangiography or other means, surgical repair may be possible. Patients with diffuse dilatation of lacteals in intestinal villi may respond to a low-fat diet; when they do, serum proteins return to normal levels and lymphocyte counts rise.<sup>217</sup> It is possible that similar results may be achieved with a medium chain triglyceride diet.<sup>180</sup>

## Immune Deficiency Associated with Lymphoproliferative Diseases and Other Malignant Conditions

Immune functions frequently are impaired in patients with malignant diseases. While the most striking defects are seen in association with tumors originating in immunologically active tissues, other malignant conditions can also be associated with poor immune responses. These abnormalities may be further aggravated by chemotherapy, radiotherapy, viral infections, and surgical procedures, all of which have been shown to be immunosuppressive. The clinical features of lymphoproliferative disorders and their management have been discussed elsewhere in this book.

### *Multiple Myeloma and Macroglobulinemia*

Since multiple myeloma (Chapter 52) and Waldenström's macroglobulinemia

(Chapter 53) arise in cell lines that ordinarily produce antibodies, it is not surprising that antibody production is impaired in patients with these malignant conditions. Because myeloma proteins offer no protection against infections, myeloma patients characteristically suffer from repeated bouts of sepsis, usually due to high-grade encapsulated organisms such as pneumococci. In this regard, the manifestations in patients with multiple myeloma resemble those in children with sex-linked agammaglobulinemia (page 1378). Patients with the greatest impairment in antibody production also suffer most frequently from infections.<sup>125</sup>

The immune deficiency of multiple myeloma is due to two factors: (1) defective antibody synthesis<sup>125,240,252,254,279,320</sup> and (2) increased rates of gamma globulin catabolism.<sup>308</sup> As a result, most patients with multiple myeloma have decreased levels of normal immunoglobulins, often less than 20% of those of controls.<sup>279,295</sup> Sometimes immunoglobulin levels return to normal following successful chemotherapy.<sup>256</sup> The response to primary antigenic challenge in patients with multiple myeloma is characterized by a prolonged induction time for IgM production and a more rapid switchover to IgG production than takes place in normal individuals.<sup>254</sup> In addition, peak antibody titers for IgM and IgG are much lower than are ordinarily found and they decline rapidly, due, at least in part, to the increased rate of gamma globulin catabolism in this disease.<sup>308</sup> In contrast to defective primary responses, secondary antibody responses are less severely affected in persons with multiple myeloma than they are, for instance, in those with chronic lymphocytic leukemia.<sup>125,240</sup>

Cellular immunity remains relatively intact. Delayed hypersensitivity reactions to natural, previously encountered antigens remain positive, and it is possible to sensitize these patients to new antigens such as KLH or DNCB,<sup>240,254,277</sup> although the responses may perhaps be weaker than in the normal person.<sup>251</sup> The rejection time for skin allografts is often delayed, but these allografts do not survive indefinitely.<sup>277</sup>

It has been reported that when lympho-

cytes from patients with multiple myeloma are cultured in the presence of PHA<sup>295</sup> or specific antigens, such as streptolysin O, streptokinase-streptodornase, and vaccinia,<sup>254</sup> their response to these stimuli is impaired. Other investigators have reported normal responses to PHA.<sup>260</sup>

Thus patients with multiple myeloma have marked defects in primary antibody production, less severe defects in secondary antibody production, and only moderate impairment of cellular immunity.<sup>249</sup>

Patients with Waldenström's macroglobulinemia suffer from defects in both primary and secondary antibody production,<sup>125,191,290</sup> but do not develop infections as readily as do patients with multiple myeloma. The response of lymphocytes to PHA is markedly impaired.<sup>295</sup>

### Hodgkin's Disease

One of the most striking immunologic features of advanced Hodgkin's disease is lymphopenia.<sup>223</sup> This generally is a poor prognostic sign carrying in its wake progressive deterioration of cell-mediated immune responses. Terminally the condition of these patients may be similar to that of children with thymic hypoplasia (page 1384). The loss of T-cell function is a progressive one that changes and deteriorates with the advancing disease process. Thus, in early localized disease, lymphocyte counts often are normal, and the incidence of positive delayed hypersensitivity reactions to commonly encountered antigens, while lower than normal, is still considerable.<sup>224,234,273</sup> Similarly, many patients with localized disease can be sensitized to contact allergens such as DNCB,<sup>234</sup> although this has been disputed.<sup>224</sup> As the disease becomes more generalized, there is a higher incidence of unresponsiveness to previously encountered antigens,<sup>270,273,307</sup> and sensitization to DNCB becomes more difficult or impossible.<sup>224</sup> Primary sensitization with BCG or other protein antigens becomes equally unsuccessful.<sup>237</sup> In addition, passive transfer of delayed hypersensitivity reactions by the use of sensitized lymphocytes from normally reactive donors becomes impossi-

ble.<sup>12,237,270,271,287</sup> This may perhaps be related to the presence of an antilymphocyte factor in the plasma of patients with Hodgkin's disease.<sup>248</sup> Indeed, some workers have demonstrated that B cells from patients with Hodgkin's disease produce large quantities of IgG with specificity for homologous peripheral blood lymphocytes.<sup>240a,277a</sup> It has been postulated that this phenomenon may be responsible for the observed immunologic defects in Hodgkin's disease.<sup>240a</sup> Skin allografts<sup>247,271</sup> and allogeneic bone marrow grafts<sup>230</sup> often survive for prolonged periods in patients with active Hodgkin's disease.

Since cellular immunity may be impaired in patients with nearly normal lymphocyte counts, it is quite unlikely that the anergy so characteristically present in patients with Hodgkin's disease is due to lymphocytopenia alone. Thus, when lymphocytes from persons with Hodgkin's disease are transferred into the skin of a normal recipient,<sup>246</sup> the expected local graft-versus-host reaction is very poor when compared to the effect of normal lymphocytes,<sup>224</sup> suggesting that lymphocytes from patients with Hodgkin's disease are intrinsically defective, even when removed from their native environment.

The lymphocytes of patients with Hodgkin's disease also do not respond normally in vitro to phytohemagglutinin, allogeneic cells, or antigen to which they have been exposed previously,<sup>222,224,234,251,262,311</sup> but care must be taken in the interpretation of these tests since their outcome is influenced by factors other than disease activity, such as radiotherapy and chemotherapy.<sup>251</sup> In addition, a factor found in the plasma of persons with Hodgkin's disease may have an inhibitory effect on lymphocyte function in vitro.<sup>311</sup>

Macrophage function is probably normal in patients with Hodgkin's disease<sup>231</sup> and reticuloendothelial phagocytosis appears to be increased.<sup>302</sup> The ability of neutrophils to kill ingested *Candida albicans*<sup>276</sup> may be decreased.

Antibody production is impaired in patients with advanced disease, but the deficit is never as pronounced as that of cellular immunity.<sup>225,234,237,296</sup> In patients with severe generalized disease, primary immuniza-

tion may lead to lower peak titers, and these may be poorly maintained.<sup>260,294</sup> In addition, the onset of detectable antibody production may be delayed and the switchover from 19S to 7S antibody formation may not occur.<sup>260</sup> Apparently, secondary antibody responses are relatively intact, even when the same antigen fails to elicit delayed hypersensitivity reactions.<sup>229</sup> Because of decreased antibody formation, hypogammaglobulinemia sometimes develops in later stages of the disease.<sup>312</sup>

Patients with Hodgkin's disease are prone to develop tuberculosis, cryptococcosis, and certain fungal and viral infections,<sup>227-270</sup> all of which are primarily controlled by cellular immunity.

### *Non-Hodgkin's Lymphoma*

Detailed studies of immune function comparable to those for Hodgkin's disease are not available for most other lymphomas.

Patients with *lymphosarcoma* may have impaired primary and secondary immune responses<sup>229-256,296</sup> and hypogammaglobulinemia may develop late in the course of the disease.<sup>280</sup> Delayed hypersensitivity reaction to microbial antigens is impaired and becomes more marked as the disease progresses.<sup>273</sup> skin allograft rejection time is prolonged,<sup>280</sup> and the *in vitro* response to PHA is depressed.<sup>289</sup> Phagocytosis generally is intact.<sup>249</sup>

Patients with *reticulum cell sarcoma* sometimes have hypogammaglobulinemia,<sup>280</sup> cutaneous anergy,<sup>257-289</sup> and delayed rejection of foreign skin grafts.<sup>280</sup> Their *in vitro* response to mitogens is often,<sup>257-289</sup> but not always,<sup>228</sup> impaired and their lymphocytes survive poorly in culture.

In patients with *Kaposi's sarcoma*, humoral and/or cellular immune responses may be impaired.<sup>240b,278</sup>

In those with *Burkitt's lymphoma*, immunoglobulin levels may be low.<sup>239-286,319</sup> In African subjects,<sup>319</sup> but not in American ones,<sup>239</sup> impaired primary antibody formation has been found. Cellular immunity generally is normal<sup>239-318,319</sup> except in patients suffering from disseminated disease.<sup>310</sup>

### *Chronic Lymphocytic Leukemia*

Small lymphocytes from patients with chronic lymphocytic leukemia (CLL) may be either normal or abnormal (leukemic). These populations of cells can be identified and separated by a number of laboratory techniques.<sup>252</sup> In CLL the clone of malignant lymphocytes is usually derived from B cells,<sup>316</sup> but a few cases of T-cell CLL have been described.<sup>242a,291a</sup> It is therefore not surprising that the most common immunologic defect in CLL is an impairment in antibody production; occasionally defects in cellular immunity can also be demonstrated (see below).

Hypogammaglobulinemia and agammaglobulinemia are frequent accompaniments of CLL.<sup>210,267,281,291,294</sup> especially in long-term survivors and in patients whose disease is widely disseminated.<sup>314</sup> With present modes of therapy the process is probably irreversible. IgM and IgA levels appear to be affected more readily than IgG levels<sup>210,297</sup> and good correlation between the degree of hypogammaglobulinemia and frequency of bacterial infections has been established.<sup>291,301</sup> Both primary and secondary antibody responses are severely impaired,<sup>240,291,301</sup> and, while patients with higher gamma globulin levels usually show better responses than those with low levels, the response of both groups is abnormal. The primary immune response in CLL is characterized by a prolonged induction time for IgM-antibody production and a delayed switchover to IgG production.<sup>290</sup>

Delayed hypersensitivity reactions to commonly encountered antigens remain intact in most patients with CLL.<sup>222,240,291,301</sup> Many of these patients can also be sensitized to DNCB,<sup>332</sup> but generally poor responses to related chemicals such as DNFB have also been reported.<sup>240</sup> Reactivity to DNCB may be transferred by lymphocytes from reactive to nonreactive patients.<sup>232</sup> DNCB nonreactors generally appear to suffer from more advanced disease than do reactors.<sup>232</sup> Some patients are unable to reject skin allografts normally.<sup>282</sup>



The leukemic lymphocytes in CLL respond poorly to general and antigenic stimulation in culture.<sup>288,303</sup> Not only is the peak response delayed, but it is also below normal.<sup>233,293</sup> The defect is most pronounced in patients with high lymphocyte counts<sup>288,293</sup>; improved responses are noted following successful therapy and lowering of lymphocyte counts.<sup>233,300</sup> Other abnormalities of cultured lymphocytes include slow formation of new ribosomes,<sup>293</sup> abnormal RNA patterns in stimulated cells, and delayed induction of RNA methylases.<sup>252</sup> Morphologic and electronmicroscopic differences have also been reported.<sup>252</sup>

Thus patients with chronic lymphocytic leukemia suffer mainly from deficits in humoral immunity and to a lesser degree from impaired cellular immunity. In view of this combined abnormality it is not surprising that new neoplasms develop in over one third of patients with CLL.<sup>264</sup>

### Acute Leukemia

Immunoglobulin levels are usually normal in patients with acute leukemia,<sup>252</sup> although unusually high levels have been reported in juveniles with myelomonocytic leukemia in whom there is a frequent tendency to homogeneity and light chain imbalance.<sup>235</sup>

Primary and secondary antibody production usually are normal<sup>241,256,274,303</sup>; changes from normal are generally attributed to the effects of therapy.<sup>231,272</sup> Often there is recovery of immunocompetence between courses of therapy.<sup>258,259</sup> Cellular immunity is intact in many of the patients<sup>211</sup> and when it is depressed during remission-induction therapy it may also be regained between treatments. The same is true for in vitro lymphocyte responsiveness to PHA.<sup>258</sup> When defective immune responses can be demonstrated, they often indicate a poor prognosis. On the other hand, a good clinical response to chemotherapy correlates well with normal delayed hypersensitivity prior to therapy, good in vitro responses of lymphocytes to PHA, and the ability to become sensitized to new antigens.<sup>261</sup> In addition, patients who

convert from immunoincompetence to competence with therapy tend to achieve remission, whereas those converting from immunocompetence to incompetence do not.<sup>261</sup> More recent studies have shown that a good prognosis also is correlated with the ability of lymphocytes to respond to autologous leukemia cells by proliferation in vitro, as well as with the presence of IgG bound to the surface of leukemic cells,<sup>250</sup> presumably representing antibody against malignant cells. Thus, in leukemia<sup>250,261,272</sup> as in solid tumors and lymphomas,<sup>238,242,306</sup> the patient's state of immunocompetence appears to be an important factor in determining the prognosis.

### Nonlymphoid Malignant Disease

In the early course of their disease, patients with solid tumors usually have normal cellular immunity,<sup>255</sup> but, as the disease progresses, general defects in cell-mediated immune functions become evident. These may include lymphocytopenia,<sup>317</sup> poor delayed hypersensitivity reactions,<sup>263,268</sup> decreased ability to reject implants of cancer cell lines,<sup>309</sup> and weak responses in lymphocyte transfer tests.<sup>255</sup> The response of lymphocytes to PHA and other stimulants in vitro may also be depressed.<sup>245,315</sup>

There appears to be good correlation between intact cellular immunity and prognosis.<sup>185,242</sup> In addition, recovery of immune functions following therapy is an omen of good prognosis and responsiveness to further therapy.<sup>238,253</sup>

For a discussion of specific tumor immunity, see page 329.

### Sarcoidosis<sup>237,265,266,275</sup>

Boeck's sarcoid, like early Hodgkin's disease, results in a puzzling dichotomy characterized by highly selective loss of the delayed type of immune response in the presence of normal serum antibody production. The anergy was originally thought to represent an exclusive impairment of tuberculin hypersensitivity, but has since been shown to involve all forms of cellular immune functions.<sup>237,265</sup>

Delayed hypersensitivity reactions to commonly encountered antigens are clearly deficient in many patients with active sarcoidosis,<sup>237,243,265</sup> but anergy in these patients is at least partially dose dependent, since higher doses of antigen will make responders out of many previously "anergic" patients.<sup>237</sup> Similarly, the use of "depot tuberculin," or the administration of steroids either systemically or at the test site, significantly increases the number of reactors among patients with sarcoidosis<sup>237</sup> but does not increase the number of reactors in the general population. Thus, anergy in sarcoidosis is a relative matter unlike the anergy of Hodgkin's disease, which cannot be improved by these procedures.<sup>237</sup> Sensitization with contact allergens such as DNCB is also impaired,<sup>237,265,269,299</sup> but responsiveness becomes nearly normal when more powerful sensitizers such as pentadecyl catechol, an extract of poison ivy, is used.<sup>265</sup>

It is possible to transfer specific delayed hypersensitivity reactions from normal individuals to anergic sarcoid patients by injecting peripheral white cells from sensitized donors intradermally and skin testing at the same site some time later.<sup>313</sup> Generalized sensitization cannot be demonstrated by this technique and reactivity is rather short-lived. Patients with Hodgkin's disease cannot be passively sensitized by similar<sup>237</sup> or other techniques (see above). Lawrence<sup>275</sup> has been successful in transferring local reactivity by injecting dialyzable extracts of leukocytes, presumably containing "transfer factor" (see page 324), but systemic sensitization resulted in only two of seven patients. He suggested that the anergy of sarcoidosis may be due to decreased production of transfer factor or faulty transmission of the message it conveys.<sup>275</sup>

Lymphocytes from patients with active sarcoidosis respond poorly to stimulation by PHA in vitro.<sup>262,263,299</sup> Intermediate activity is seen when patients are in clinical remission.

The level of anergy can fluctuate. Thus conversion from an originally positive status to one of nonreactivity may take from two to eight months as the disease develops.<sup>237</sup> Conversely, reversion to tuberculin reac-

tivity has been observed upon spontaneous remission of the disease or after therapy.<sup>237</sup>

Elevated serum globulins are found in a third of patients with sarcoidosis<sup>265</sup> and circulating antibody production is either unimpaired or even better than normal.<sup>237,265</sup>

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## Disorders Primarily Involving the Spleen

### Causes of Splenomegaly

### Chronic Congestive Splenomegaly

### Concept of "Hypersplenism"

### Methods of Evaluating Suspected Splenomegaly

### Indications for Splenectomy

**I**n the preceding chapters a number of conditions have been considered in which enlargement of the spleen occurs. There remain several that have not as yet been discussed systematically. The following pages are devoted to a classification of splenomegaly, discussion of those disorders of the spleen that have not been considered elsewhere, and consideration of methods that are useful in evaluating splenomegaly. Indications for splenectomy also will be summarized.

### Frequency of Splenomegaly and Its Significance

How important is the finding of a spleen that is just palpable on physical examination? This and questions concerning the frequency of palpable spleens and their significance are raised from time to time. In one large series of 2274 private patients seen in the United States a palpable spleen was detected in 5.6%, and in 41% of these no adequate explanation was uncovered.<sup>2</sup> In another series of 5880 unselected clinic patients, only 2% had a palpable spleen and in 25% of these no cause

was found.<sup>5</sup> Unfortunately, in these two series, no follow-up was reported. In a group of 2200 students entering college, however, spleens were palpable in 2.86% and in about 30% of these the spleen was still palpable three years after initial detection.<sup>3</sup> No evidence of increased prevalence of disease was found among members of this group.<sup>3</sup> In contrast, in certain tropical countries an incidence of splenomegaly as high as 60 to 70% has been reported.<sup>4,5</sup>

Although a palpable spleen is not necessarily enlarged<sup>1</sup> and an enlarged spleen may have no serious significance, splenomegaly may be the first and only sign of disease. Therefore, it is good practice to regard a palpable spleen as a physical sign of importance and to make an effort to discover the cause. Methods for assessing splenic size and evaluating the significance of a palpated spleen are described on page 1419.

### Causes of Splenomegaly

An enumeration of the causes of splenomegaly touches practically all the types of disease to which man is heir: infectious, metabolic, circulatory, endocrine, and neoplastic, as well as purely mechanical disorders (Table 45-1). The relative frequency of these disorders as a cause of splenomegaly differs with geographic locale, socioeconomic conditions, and other factors. Thus, even in two large series from the United States, the types of

disease associated with splenomegaly differed considerably.<sup>2,5</sup>

The ensuing discussions follow the classification outlined in Table 45-1.

# 1. Inflammatory Splenomegaly

A. The "acute" enlargement of the spleen ("acute splenic tumor") that develops in association with various infections or inflammatory processes is thought to result from an increase in the defensive activities of this organ. Increased need for clearance of particulate matter from the blood (bacteria, protozoa, damaged cells, etc.) may lead to increased numbers of reticuloendothelial cells in the spleen and/or stimulate antibody production with resulting lymphoid hyperplasia. The functions of the spleen were discussed in Chapter 8.

B. Of the infectious causes of splenomegaly, those that are chronic in nature are of chief interest here since their clinical picture may resemble that of various hematologic disorders.

1. *Tuberculous splenomegaly*, so-called "primary tuberculosis of the spleen," is a very rare condition and may be confused with tuberculosis of the spleen accompanying generalized tuberculosis.<sup>11,12</sup> About 100 cases of presumed primary tuberculosis of the spleen with splenomegaly have been reported.<sup>10</sup> The symptoms include weakness, lassitude, loss of weight, a variable amount of fever, enlargement of the spleen, which may be very great, and, in some cases, hematemesis, ascites, jaundice, and purpura. Anemia and leukopenia are the most common blood changes, but there may be thrombocytopenia as well, or thrombocytopenia may occur alone; even polycythemia has been reported.<sup>11</sup> Normoblasts may be found in the blood smear in the absence of anemia. The tuberculosis may be discovered only following splenectomy performed for the treatment of what had been thought to be "Banti's disease." In most of the cases studied adequately, tuberculous lesions were found to exist elsewhere as well.<sup>13</sup> The roentgeno-

graphic demonstration of areas of calcification in the spleen is helpful in diagnosis, but such calcification has not always been evident.<sup>13</sup> In some subjects, complete relief of symptoms followed splenectomy.<sup>10</sup>

2. Splenomegaly may occur in association with syphilis, especially congenital syphilis. The clinical picture is one of splenomegaly, chronic anemia, and, usually, leukopenia. Antiluetic therapy<sup>15</sup> may or may not be helpful and, in one of the writers' patients, recovery took place only following splenectomy. Splenomegaly may also develop in patients with tertiary syphilis as the result of the formation of huge gummas<sup>16</sup> or may result from amyloidosis.

3. Splenomegaly, chronic arthritis, anemia, and leukopenia occurring in adults comprise the disease known as *Felty's syndrome*.<sup>21</sup> This syndrome resembles Still's disease of children and has also been referred to as Chauffard-Still's disease. Loss of weight and progressive weakness are manifest. Brownish pigmentation may be observed on the exposed skin. There may be generalized lymphadenopathy and the liver may be enlarged. The anemia is moderate in degree and normocytic.

Rarely severe hemolytic anemia with shortened red cell survival has been reported.<sup>23,29</sup> Leukopenia may be pronounced ( $0.8$  to  $4.2 \times 10^9$  cells/l, average 2.5 or even less) but the differential count varies. Usually there is neutropenia and lymphocytopenia, while eosinophils and monocytes may be normal or increased in number.<sup>27</sup> Neutrophil kinetic studies have demonstrated a short  $1\frac{1}{2}$  of labeled cells in the blood and subnormal marrow granulocyte reserves.<sup>20,32</sup> findings compatible with the concept that the neutropenia is due to increased cell destruction plus inadequate marrow compensation. The leukopenia was cyclic in one patient.<sup>26</sup> Asymptomatic, moderate thrombocytopenia is common. The bone marrow may show myeloid hyperplasia, "maturation arrest" with an absence of segmented neutrophils, or it may be normal.

Felty's syndrome is thought to be a variant of rheumatoid arthritis.<sup>22,27,31</sup> Splenomegaly

**Table 45-1. Classification of Splenomegaly**

- 
- I *Inflammatory splenomegaly*
    - A Acute and subacute
      - 1 Acute splenic tumor of various infections (typhoid, septicemia, etc)
      - 2 Abscess of spleen
      - 3 Infectious mononucleosis
      - 4 Subacute bacterial endocarditis
    - B Chronic
      - 1 Tuberculosis
      - 2 Syphilis, especially congenital
      - 3 Felty's syndrome, rheumatoid arthritis
      - 4 Malaria
      - 5 Leishmaniasis
      - 6 Trypanosomiasis
      - 7 Amazonian, Bengal, and American splenomegalies Histoplasmosis
      - 8 Schistosomiasis
      - 9 Echinococcosis
      - 10 Boeck's sarcoid
      - 11 Beryllium disease
  - II *Congestive splenomegaly* ( Banti's disease " " splenic anemia )
    - A Cirrhosis of the liver
    - B Thrombosis, stenosis, or cavernous transformation of the portal vein
    - C Thrombosis or other forms of obstruction of the splenic vein
    - D Less common and unrecognized causes of congestive splenomegaly
    - E Cardiac failure (occasionally)
  - III *"Hyperplastic" splenomegaly*
    - A Frankly hemolytic anemias of various types
    - B Chronic anemias with moderately increased or no increase in blood destruction
      - 1 Pernicious anemia and related macrocytic anemias
      - 2 Thalassemia, hemoglobin C disease, and combinations of Hb's C, D, E  
Lepore, or sickle cell hemoglobin and thalassemia
      - 3 Myelophthisic anemia, myelodysplasia, "aleukemic megakaryocytic  
myelosis," "agranulocytic myeloid metaplasia," etc
      - 4 Hemolytic disease of the newborn
      - 5 Systemic lupus erythematosus
    - C Thrombocytopenic purpura
    - D Benign lymphatic hyperplasia—Graves' disease
    - E Polycythemia vera
    - F "Primary splenic neutropenia," "primary splenic panhematopenia"
    - G Cryptogenic, tropical splenomegaly ("big spleen syndrome")
  - IV *"Infiltrative" splenomegaly*
    - A Gaucher's disease
    - B Niemann-Pick's disease
    - C Amyloidosis
    - D Diabetic lipemia
    - E Gargoylism
  - V *Cysts and neoplasms*
    - A True cysts (epithelial, endothelial, or parasitic; dermoids, lymphangiomas, hemangiomas, hydatid)
    - B False cysts (hemorrhagic, serous, inflammatory, degenerative)
    - C Hamartomas
    - D Leukemias
    - E Hodgkin's disease
    - F Sarcomas, primary or secondary (lymphomas)
    - G, Histiocytosis X (Schüller-Christian and Letterer-Siwe diseases)
    - H Metastatic neoplasms
-

is known to occur in 1 to 21% of persons with chronic arthritis.<sup>25</sup> The degree of rheumatoid activity is not directly correlated with the severity of the hematologic changes in Felty's syndrome. In fact, the arthritis often may have been of long duration and may have run its course and become quiescent when the syndrome has developed. Antinuclear antibodies often are found in high titer.<sup>31</sup> The splenomegaly is of mild to moderate degree (splenic weight, 260 to 2070 g, average 910) although occasionally the spleen is huge (2400 g).<sup>29</sup> Splenectomy has produced benefit (increased neutrophil counts and decreased incidence of infection) in some of the patients so treated, but the improvement is of variable duration and neutropenia may recur.<sup>20,22,30,31,32</sup> Corticosteroids are usually ineffective in relieving neutropenia.<sup>27</sup>

4. *Malaria* is a well known and common cause of splenomegaly in many tropical countries.<sup>31,38,39,43,45</sup> Patients with chronic cases<sup>38</sup> may not have jaundice even when they have substantial anemia; leukopenia and thrombocytopenia are present in a proportion of these patients.<sup>43,45</sup> Parasites may be difficult to discover. Malarial parasites harbored in the spleen are sometimes forced into the circulation by the subcutaneous injection of epinephrine. Liver biopsy may be helpful in determining the diagnosis.<sup>39</sup> The spleen is large and may be huge.<sup>40</sup> On cut section it is slate blue<sup>36</sup> and marked reticulum cell hyperplasia is noted.<sup>40</sup> The associated anemia is due largely to red cell sequestration in the large spleen as well as increased plasma volume; the red cell mass often is normal even when red cell survival is somewhat shortened.<sup>43,45</sup> A high titer of malarial antibody often is present,<sup>45</sup> and a high incidence of cold hemagglutinins with anti-i specificity has been found.<sup>41</sup> Treatment for malaria has produced benefit in some patients<sup>35,45</sup> but not others.<sup>43</sup> Splenectomy also has been beneficial, according to some reports,<sup>45</sup> but this operation is advised only for patients with disabling symptoms since even massive splenomegaly seems to be well tolerated by many subjects.<sup>43</sup> There is evidence that, in the

spleen, malarial parasites are removed from red cells by "pitting."<sup>46</sup>

5. Both the Asiatic<sup>51</sup> and the Mediterranean<sup>52</sup> or infantile form of *leishmaniasis* (*kala-azar*) produce splenomegaly of extraordinary degrees, irregular fever, anemia, and leukopenia. Lymphadenopathy may be present, even as the primary and most important symptom.<sup>50</sup> The anemia is normocytic and the leukopenia is due to a reduction in all types of cells, especially neutrophils.<sup>51</sup> The urobilinogen content of the urine is increased. The diagnosis is made by the demonstration of the protozoan, *Leishmania donovani*, by means of splenic or bone marrow puncture<sup>51,52</sup> (Fig. 45-1).

6. In both the African and the American forms (Chagas' disease) of *trypanosomiasis* the spleen is usually enlarged, but rarely is the enlargement pronounced.

7. A clinical picture resembling that of kala-azar has been observed in the region of the Amazon River in South America<sup>50</sup> and in Bengal (India)<sup>36</sup> as well as in Central America and the United States. *Bengal splenomegaly* is attributable to a number of factors, including malaria, malnutrition, post-necrotic cirrhosis of the liver, and, ultimately, the development of portal hypertension.<sup>53</sup> The *American splenomegalies*<sup>55</sup> have been ascribed to *Histoplasma capsulatum* of Darling, a fungus morphologically resembling *Leishmania donovani*<sup>57</sup> (Fig. 45-1). *Histoplasmosis* has been reported in Europe<sup>56</sup> and in Africa<sup>58</sup> as well as in the American continent.

8. Infestation with the flukes, *Schistosoma mansoni* or *S. japonicum*, (*schistosomiasis*, *Egyptian splenomegaly*) results in diffuse hyperplastic periportal cirrhosis of the liver, perisplenic vein fibrosis, and thrombosis within the splenic vein. Progressive splenomegaly is the consequence. The liver is at first enlarged, later it shrinks and ascites develops. Eosinophilia may occur in the earliest stage of the disease; anemia and leukopenia, later.<sup>62,63</sup> The symptoms are produced by the deposition of the lateral-spined ova in the proximal and distal peripheral capillaries of



Fig 45-1. 1, *Leishmania donovani* in a large endothelial cell. Obtained by sternal puncture in a patient with kala azar. 2, *Histoplasma capsulatum* from the spleen of a patient with histoplasmosis. Note the characteristic saucer shape of the fungus, engulfed by phagocytes. 3, *Toxoplasma cuniculi* from the spleen of a rabbit. Two lymphocytes and an endothelial cell are also shown. 4, *Piroplasma* in red cells (cattle). 5, *Bartonella muris* in the blood of a rat. The organism is sometimes bacilliform, sometimes coccoid.

the portal system, with resulting fibrosis.<sup>63</sup> Differentiation from other forms of splenomegaly presenting as the "Banti syndrome" depends chiefly on the demonstration of the ova in the stools. The ova are rarely found in the spleen. Schistosomiasis has been ob-

served not only in the Nile region<sup>62</sup> and in China and Japan, but also in coastal East and West Africa, throughout the Amazon Basin, and in parts of northern South America and southern Central America as well as in Puerto Rico.

9. Splenomegaly may follow *echinococcus* disease of the liver. Echinococcus cysts of the spleen are rare.<sup>70</sup>

10. In *sarcoidosis* the spleen may be slightly, moderately, or even greatly enlarged, and sarcoidosis of the spleen, with only insignificant or absent lesions elsewhere, also has been described.<sup>71</sup> In a small percentage of the 20% of patients with sarcoidosis in whom splenomegaly occurs, thrombocytopenia is found most frequently, but also hemolytic anemia, neutropenia, pancytopenia,<sup>72</sup> and splenic rupture have been observed.

## II. Congestive Splenomegaly

Congestive splenomegaly will be discussed separately (page 1412).

## III. "Hyperplastic" Splenomegaly<sup>51</sup>

Enlargement of the spleen is a frequent finding in patients with *anemia*. It is noted in those with acute anemia as well as in those with chronic anemias of various types (Table 45-1) and in some patients with thrombocytopenic purpura. It has even been reported, apparently as a result of hemolysis, in chronic phenacetin users.<sup>73</sup> As a rule, in none of these subjects does the splenomegaly assume great proportions. Small foci of extramedullary blood formation may be found in the spleens of many of these patients. In patients with *myelofibrosis* and in a number of those with *myelophthisic anemia*, splenomegaly may be the outstanding evidence of disease (Chapter 57).

The splenomegaly associated with *polycythemia vera* (Chapter 30) can also be classed under the heading of "hyperplastic splenomegaly."

Several syndromes of unknown cause are probably best classified among the "hyperplastic" splenomegalies. "Primary splenic neutropenia" was described by Wiseman and Doan as being characterized by fever, pain in the splenic region, and splenic enlargement, with essentially normal or somewhat hyperplastic bone marrow.<sup>198</sup> It has been

reported more often in females than in males and, in the few cases described,<sup>170,185,193,196</sup> the manifestations were acute, subacute, or chronic and, in one instance, recurrent. Associated infections, especially oral ulcerations, have been common.<sup>170</sup> The splenomegaly ranged from slight to marked; the spleen was palpable in 72% of one series of 25 patients.<sup>170</sup> Thrombocytopenia and hepatic dysfunction were observed in one patient.<sup>193</sup> Relief of all symptoms was reported to follow splenectomy and relapse occurred in only one of 25 subjects.<sup>170</sup> However, the duration of follow-up was not clearly stated. The removed spleens showed extreme histiocytic predominance (clasmatoctytosis), the majority exhibiting excessive phagocytosis of granulocytes. Consequently, the disorder was attributed to excessive lysis of neutrophils by the spleen.<sup>187</sup>

"Primary splenic panhematopenia"<sup>168,169</sup> and "nontropical idiopathic splenomegaly"<sup>165</sup> may be closely related to "primary splenic neutropenia" and, like the latter condition, are of unknown cause. The clinical manifestations, as reported, have been somewhat vague and variable and may have been present for a few weeks to many years. In a few subjects, they were described as recurring periodically. Complaints included lassitude, palpitation, fever, and vague aches or pains in the extremities. Oral ulceration and indolent ulcers on the lower extremities were noted in some of these patients. Splenomegaly was prominent in several series,<sup>165,180</sup> but was noted in only three of 17 of Doan's patients.<sup>170</sup> Lymphadenopathy has usually been minimal or absent, but we and others<sup>165</sup> have seen patients who were initially thought to have a lymphoma. The reticulocyte percentage was slightly or greatly increased in some patients but not in others.<sup>170</sup> There has been little or no other evidence of increased blood destruction.<sup>180</sup> Polychromatophilia or increased osmotic fragility of the red cells was sometimes found.<sup>169</sup> The bone marrow was hyperplastic. A prompt response to splenectomy was reported in 16 of 17 patients, but, in 3, relapse occurred later and after 8 to 10 years only 8 remained alive and well.<sup>170</sup> In another series,<sup>180</sup> improvement after sple-

nectomy tended to be gradual rather than immediate and complete.

For the diagnosis of primary splenic neutropenia and panhematopenia, much stress has been laid on the *adrenalin test*.<sup>169</sup> This test, however, is regarded as unreliable by most investigators.<sup>160,180</sup> Results similar to those described in patients with primary splenic neutropenia have been observed in normal individuals and even in persons who have undergone splenectomy. As to increases in the number of neutrophils following the administration of epinephrine it is now well established that these are attributable to a shift of cells from the marginal to the circulating pool of granulocytes (Chapter 6).

It is noteworthy that most cases of primary splenic neutropenia and panhematopenia were reported several decades ago when diagnostic methods were fewer and less helpful than they are now. It must be admitted, however, that cases of splenomegaly associated with neutropenia or pancytopenia which defy diagnosis are still encountered occasionally. The pathogenesis of primary splenic neutropenia and of primary splenic panhematopenia as examples of "primary hypersplenism" is discussed on page 1319.

Splenomegaly of obscure etiologic background is relatively frequent in the *tropics*. A syndrome of enlargement of the spleen, often massive, has been found among the Chinese of Hong Kong and in parts of China and Formosa ("cryptogenic splenomegaly of China").<sup>76</sup> This syndrome has been associated with varying degrees of liver damage, marked by features of both diffuse hepatic fibrosis and post-necrotic scarring. In some subjects the splenomegaly seemed to anticipate the liver changes. Abnormal vascular fragility, unrelated to thrombocytopenia, and chronic leg ulcers are additional features of this disorder. The associated anemia was thought to be due to both the destructive action of the spleen and abnormality of the red corpuscles, the latter possibly caused by the liver disease (page 708). Expanded plasma volume also contributed to the anemia. All of these manifestations were reported to respond favorably to splenectomy.

The "tropical splenomegaly" or "big spleen"

*syndrome*<sup>84,190</sup> is characterized by massive splenomegaly of uncertain cause. It is said to be found only where malaria is endemic. A history of recurring fever is common, but, once splenomegaly has developed, patients have usually been afebrile. Many have been only mildly incapacitated despite massive spleens extending into the pelvis. Anemia, attributed to increased plasma volumes and a greater splenic red cell pool, and leukopenia and thrombocytopenia are common.<sup>84</sup> Malarial infection may play an etiologic role, the syndrome possibly resulting from an atypical immune response to malaria.<sup>84</sup> Malaria chemotherapy, if continued for long periods, may result in decrease in spleen size and increase in hemoglobin level. Splenectomy has been beneficial in selected patients.<sup>84,190</sup>

#### IV. "Infiltrative" Splenomegaly

Under this designation may be grouped those instances of splenomegaly apparently resulting from excessive storage of normal and abnormal metabolic products—a cerebroside in Gaucher's disease and sphingomyelin in Niemann-Pick's disease. Perhaps gargoylism should be added here. These conditions were discussed in Chapter 42. The splenic enlargement found in amyloidosis, glycogen storage disease, and in diabetic or other forms of *lipemia* also may be added to this group of splenomegalies. In one patient with type I hyperlipoproteinemia, the spleen contained large amounts of ceroid.<sup>80</sup>

#### V. Cysts and Neoplasms

Tumors of the spleen may be benign or malignant. The benign tumors include cysts and hamartomas. Splenic cysts may be lined by a specific secreting membrane (true cysts); these are epithelial (dermoid, epidermoid), endothelial (lymphangioma, hemangioma, polycystic, serous), or parasitic (echinococcus).<sup>85</sup> False cysts may be hemorrhagic,<sup>96</sup> serous, or inflammatory, or they may be due to degenerating liquefaction of areas infarcted<sup>95</sup> by embolism or arterial thrombosis. Over 400 nonparasitic cysts have been re-

ported.<sup>92</sup> Fullness in the epigastrium, vague digestive complaints, or even epigastric pain may develop and, when the diaphragm has been elevated, dyspnea, cough, and pain radiating to the shoulder may be experienced.<sup>83</sup> The tumor may be felt in the left upper quadrant as a rounded mass that displaces the stomach downward and to the right. The splenic flexure of the colon and the left kidney may be displaced downward. Calcification is not common. Nonparasitic splenic cysts have been noted most frequently in women, especially in those of childbearing age. Their cause is unknown, but trauma has been thought to play a dominant role.

Hemangioma of the spleen does not usually produce a tumor that is detectable clinically, but it may rupture into the peritoneal cavity, in which instance the diagnosis of perforated peptic ulcer or acute appendicitis may be mistakenly made.<sup>97</sup> Hemangiosarcoma is rare and may be associated with a leukoerythroblastic blood picture.<sup>67</sup>

Hamartomas are rare benign tumors composed of abnormal mixtures of normal splenic elements.<sup>99</sup>

The splenomegaly of Hodgkin's disease is discussed in Chapter 50; histiocytosis X, in Chapter 42. Sarcomas of the spleen most often represent only one aspect of more widespread disorders (lymphosarcoma, follicular lymphoma, reticulum cell sarcoma, Hodgkin's sarcoma). These diseases are discussed in Chapter 51. Primary sarcoma of the spleen, a tumor arising from the supporting tissues of the organ, is relatively rare. In addition to a palpable mass in the left upper quadrant of the abdomen, there may be pain in this area, and fever, anemia, and general debility may be noted. More rarely, pleural effusion, gastrointestinal hemorrhage, and splenic rupture have been observed.<sup>91, 98</sup>

Of metastatic tumors, carcinoma is the most frequent type.<sup>90</sup> Although it is unusual for the spleen to be sufficiently enlarged to be palpable clinically, metastases to the spleen, from lung, breast, skin, and cervix, have been observed at autopsy in 2.3% of patients with carcinoma.<sup>80</sup> The low incidence of splenic metastases as compared with me-

tastases found in lymph nodes, liver, and lungs has been attributed to inequality of exposure. Lymphatic spread of carcinoma to the spleen has been observed.<sup>88</sup> No inhibiting effect of splenic tissue on carcinomatous growth has been demonstrated.

## Chronic Congestive Splenomegaly

("Banti's Disease," "Splenic Anemia")

### History and Definition

In 1866<sup>114</sup> the term "splenic anemia" was introduced to designate cases of anemia associated with splenomegaly which could not be classified as frank leukemia. At the end of the century, Banti<sup>101</sup> described a form of splenomegaly that was not associated with leukemia, Hodgkin's disease, or hemolytic jaundice, nor was it caused by malaria, syphilis, or other recognized diseases. He laid down clinical and histologic criteria for the recognition of the disorder and described three stages: (1) an anemic phase with splenomegaly, leukopenia, asthenia, and occasional hemorrhagic episodes; (2) a later intermediary phase in which hepatic enlargement occurred, urobilinuria developed, and a dirty, brownish discoloration of the skin sometimes appeared; and (3) a final stage of liver atrophy and ascites. The spleen was markedly enlarged and histologically was characterized by conspicuous thickening of the fibrillar reticulum. Banti considered the spleen to be the primary seat of the disease and assumed that anemia, hepatic cirrhosis, and sclerosing endophlebitis of the splenic and portal veins followed.

Osler<sup>132</sup> drew the attention of English-speaking clinicians to the condition and seemed to accept it at its face value, but many pathologists refused to endorse the concept enunciated by Banti. Dürr,<sup>108</sup> for example, showed that sections of spleens from Banti's own subjects could not be differentiated from those found in the spleen in patients with hepatic cirrhosis. Eppinger<sup>110</sup> introduced the term "hepatolienal fibrosis" to describe the



essential pathologic changes.<sup>127</sup> Later investigations of the pathogenesis of the disorder showed that this syndrome is associated with portal hypertension and led to the use of the term "chronic congestive splenomegaly."<sup>149</sup>

### Clinical Features

The concept that Banti's disease is a specific entity is now largely discredited, but the syndrome of congestive splenomegaly is recognized. The clinical features of the syndrome, as described in the early reports, were as follows.

Those affected were stated to be usually under 35 years of age, the disease occurring even in children.<sup>143</sup> Females were twice as numerous as males in Banti's series and this was true of Giffin's series,<sup>113</sup> but was not the case in all series reported.<sup>109,127</sup> A familial incidence, though unusual, was not unknown.<sup>127</sup> The onset was described as usually insidious, but might come with explosive suddenness characterized by vomiting of blood or the passage of large, tarry stools. Gastrointestinal hemorrhage occurred in about half the patients.<sup>128</sup> In others, abdominal pain or discomfort, the discovery of a mass in the left upper quadrant of the abdomen, or symptoms of weakness and general lassitude referable to anemia were the manifestations responsible for consulting a physician. Flatulence, diarrhea, vague indigestion, or even mild jaundice in association with abdominal pain and fever and a non-icteric type of sallow-brown pigmentation of the skin were described. Epistaxis occurred in about a third of the patients, but other hemorrhagic manifestations such as purpura were unusual. The spleen was described as large and might be enormous, extending even to the pelvic brim.<sup>113</sup> Moderate hepatic enlargement was observed at an early stage in perhaps a third of the subjects.<sup>104</sup> Lymphadenopathy was absent and weight loss was not conspicuous.

Splenomegaly sometimes preceded anemia<sup>113</sup> and the latter, unless hemorrhages had occurred, was at most moderate in degree and as a rule normocytic in type.<sup>104,122</sup> The average red cell count in 151 reported cases was

$3.4 \times 10^{12}/l$ . At this stage the morphologic appearance of the red cells was not remarkable. Repeated hemorrhages resulted in hypochromic microcytic anemia with all the morphologic features of this type of anemia (page 657). Following a hemorrhage the reticulocytes might be slightly or moderately increased in number and occasional normoblasts were observed. The fragility of the red corpuscles to hypotonic saline solutions was normal or reduced.<sup>128</sup> When the condition was of long standing and well marked, cirrhosis of the liver was present. Macrocytic anemia sometimes developed temporarily following an acute hemorrhage.<sup>150</sup>

Leukopenia is the most constant feature of the blood picture. Leukocyte counts less than  $5.0 \times 10^9/l$  were observed in about two thirds of the patients.<sup>113,128</sup> A curious feature of the leukopenia is that the diminution often affects all the types of cells, the ratio of the polymorphonuclear cells to the lymphocytes and monocytes remaining normal as a rule. Leukocytosis may follow a severe hemorrhage or may be associated with venous thrombosis or an acute exacerbation of hepatitis.

The platelet count is often somewhat reduced and sometimes is well below  $100 \times 10^9/l$ . The bleeding time may be prolonged.<sup>128</sup> As a rule, however, bleeding and coagulation times are normal.

Findings in the *bone marrow* on sternal puncture vary. In the earlier stages, at the time when there is little or no anemia, no abnormality may be noted. Myeloid hyperplasia in the earliest stage<sup>123</sup> and "maturation arrest" of the myeloid and megakaryocytic tissue later have been described. In the last stage in which cirrhosis of the liver is present, there may be normoblastic hyperplasia.

The relation of these changes in the blood and bone marrow to the syndrome of "hypersplenism" will be discussed shortly (page 1417).

### Course

The course of the disease was described as prolonged and often benign. Sometimes it

seemed to become arrested spontaneously, the patient living for a number of years with little or no disability. At any time, however, the uneventful course might be interrupted by one or more episodes of gastrointestinal hemorrhage, portomesenteric venous thrombosis, or hepatitis. Other patients experienced more or less steady progression of the condition, with the development of symptoms of hepatic insufficiency and the signs of portal venous obstruction. Death frequently resulted from one of the above-mentioned complications or from intercurrent unrelated disease.

### Diagnosis

Moderate anemia and leukopenia are found in association with many conditions producing splenomegaly and the presence of any of these must be ruled out before the diagnosis of congestive splenomegaly is considered. Hemolytic anemias, "aleukemic" leukemia, chronic hypochromic anemia, thalassemia, and hookworm anemia, as well as most of the other conditions listed in Table 45-1, have been mistaken for "Banti's disease." The finding of lymphadenopathy or of immature leukocytes in the blood smear favors conditions other than congestive splenomegaly. Sternal marrow examination is useful in ruling out the possibility of "aleukemic" leukemia.

Chronic congestive splenomegaly may result from any one of a variety of causes. The splenic vein may be obstructed because of thrombosis or as the consequence of compression from pancreatic fibrosis or aneurysm of the splenic artery, or by a tumor of the pancreas, such as a cystadenoma<sup>115</sup> or carcinoma.<sup>126</sup> The portal vein may be obliterated due to bland thrombosis, pylephlebitis, or from cavernous transformation, or it may be absent ("aplasia of portal vein").<sup>140</sup> The hepatic veins may be thrombosed (Budd-Chiari syndrome) and cause increased vascular resistance. The obstruction, on the other hand, may be intrahepatic and result from cirrhosis. The cirrhosis may be of the Laennec variety or may follow hepatitis or schistosomiasis. However, in a few reported cases

it was not possible to demonstrate either intra- or extrahepatic obstruction.<sup>147</sup> In these it was postulated that the portal hypertension was caused by increased blood flow through the portal vein.

Extrahepatic portal obstruction is more common in patients under 18 years of age than later in life. There are likely to be no gastrointestinal symptoms except when bleeding occurs. The liver is not palpable. Splenomegaly may be associated with the Cruveilhier-Baumgarten syndrome, which is recognized by the presence of a prominent para-umbilical vein, as well as a venous hum and a thrill at the site of the para-umbilical circulation.<sup>105,119</sup>

A variety of relatively simple procedures are available for the study of patients with congestive splenomegaly. Liver function studies obviously are necessary. It is important to note, however, that reduced Bromsulphalein excretion may be due to diminished blood flow through the liver, and that portal hypertension may be responsible for abnormal findings in other liver function tests, even when the obstruction is extrahepatic.<sup>135</sup> The extent of collateral circulation should be determined and esophageal varices looked for. The portal venous pressure may be gauged by measurement of intrasplenic pressure by means of percutaneous splenic puncture with a fine needle and a strain gauge.<sup>100</sup> Intrasplenic pressure has been found to bear a linear relationship to the portal venous pressure. Before making a decision concerning treatment, portal venography should be carried out. This may be accomplished by the percutaneous injection of the splenic pulp with radiopaque solutions,<sup>211</sup> or by direct injection of a contrast medium into the portal vein at operation.<sup>211</sup> The latter is necessary to confirm a diagnosis of anatomic obstruction, as the portal vein sometimes is not completely opacified in the percutaneous splenoportogram.

### Treatment

The chief problem is to reduce the portal hypertension and thus lessen the chance of hemorrhage. Unless this can be accomplished

the patient has little chance of survival beyond a year or two at the most.<sup>140</sup>

If iron-deficiency anemia is present due to blood loss, iron therapy (page 660) is indicated. There is no need for administration of folic acid or vitamin B<sub>12</sub>, but the diet should be nourishing and contain an adequate supply of vitamins. Severe hemorrhage will necessitate blood transfusions and balloon tamponade may be required if early severe bleeding persists.

Omentopexy,<sup>120</sup> the production of an Eck fistula, and, later, splenectomy were employed in attempts to relieve the portal hypertension. *Splenectomy* may relieve the leukopenia or pancytopenia,<sup>125</sup> when present, and is effective in relieving obstruction close to the splenic hilum, but otherwise it has not proved to be useful since little reduction in portal hypertension results. The immediate postoperative mortality has been high and hemorrhage has recurred in as many as 50% of the patients.<sup>125</sup> In a comparative study of 51 splenectomized patients and 43 who were not subjected to splenectomy, no differences between the two groups could be demonstrated in regard to life expectancy, prevention of the progress of hepatic disease or anemia, or the occurrence of hematemesis.<sup>118</sup> The implications of another study were similar.<sup>116</sup> Many surgeons believe that a splenectomy should not be attempted unless one is prepared to perform a venovenous anastomosis, since this may be the only opportunity to construct a satisfactory shunt.<sup>124,139</sup>

Disappointment with the results of splenectomy led to the introduction of *portal-systemic shunt* operations for the purpose of relieving portal hypertension.<sup>124</sup> When there has been marked splenomegaly and a large-caliber splenic vein has been available, or when the portal vein has been obliterated, splenectomy and splenorenal shunt have been recommended by some, in preference to portacaval anastomosis, because of a lower incidence of severe neurologic disturbances and ammonia intoxication.<sup>103</sup> However, portacaval shunt has been the preferred procedure, on the assumption that the use of the largest-caliber vessel available is likely to maintain a patent shunt most efficiently.<sup>140</sup>

Portal-systemic shunts are advised only: (1) in patients in whom upper intestinal hemorrhage is severe and in whom the portal hypertension is due to extrahepatic block; or (2) in patients with portal hypertension associated with cirrhosis of the liver in whom there is bleeding from esophageal varices and yet minimal or absent ascites and icterus, and a reasonable degree of hepatic reserve. When these criteria were followed, the occurrence of bleeding was markedly reduced and life was prolonged.<sup>111</sup> Operative mortality was reported to be 11% and the overall mortality 29%. There is little justification for prophylactic portal-systemic shunts.

More recent experience with side-to-side portacaval anastomosis (in contrast to end-to-side) has resulted in operative mortality of only 6 to 7%, and survival curves leveling off at about 60% after three years.<sup>102</sup> In occasional patients, however, progressive splenomegaly and cytopenia have developed after portacaval shunt operations.<sup>137</sup> The use of supradiaphragmatic splenic transplantation has been successful in the management of occasional patients having portal hypertension, especially those with hepatic vein thrombosis.<sup>144</sup> This procedure apparently results in very effective shunting of blood through anastomoses between the spleen and the pleural vessels.

Marked deposition of hemosiderin has been observed on microscopic study of cirrhotic human livers after surgical portal-systemic shunting.<sup>107</sup> Experimental studies suggest that hematopoiesis generally is depressed as a result of this procedure and that iron accumulates in the liver as a consequence.

### Pathology

As a rule the spleen weighs 600 to 1200 g<sup>127</sup> but may be as heavy as 5000 g.<sup>113</sup> The capsule is thickened and adhesions between the spleen and the stomach or diaphragm may be present. On section the spleen appears grayish-red in color and firm in consistency. The trabeculae are prominent and the malpighian bodies are inconspicuous. Fibrosis, dilatation of the sinuses, hyaline degenerative

changes in the malpighian bodies and the follicular arterioles, and periarterial hemorrhages are found on microscopic examination. Siderotic nodules consisting of crystalline and amorphous iron pigments deposited in the fibrous tissue around the arterioles are seen in many instances.<sup>127</sup>

Surgeons describe distended and tense venous radicals in the splenic pedicle and rich collateral veins enveloping the spleen, as well as great distention of the splenic vein.<sup>139</sup> Endophlebitis of the splenic and portal veins is frequent, and there may be thrombosis of these veins, stenosis or cavernous transformation of the portal vein,<sup>139</sup> or compression of the splenic vein by tumors or scars. Cirrhosis of the liver may be evident or may be discovered only at microscopic examination.

### Pathogenesis

The histologic picture<sup>81,127</sup> in the spleen, as described by Banti, is not specific but may be seen, for example, in patients with cirrhosis of the liver and in those with portal or splenic vein thrombosis. The changes are of such a nature as might easily be produced by congestion of the spleen.<sup>127</sup> The distention in the sinuses, the hemorrhages, fibrosis, and also the siderotic nodules can be explained on this basis. In addition, definite signs of portal venous congestion are usually observed. Measurements of the pressure in the splenic vein in 14 patients presenting the clinical picture of Banti's syndrome showed the pressure to range from 225 to more than 500 mm normal saline solution and was greater than 300 in 11 of the subjects.<sup>139</sup> In contrast, in 15 patients having splenomegaly of other types, such as hemolytic disease and thrombocytopenic purpura, the pressure ranged from 70 to 275 mm in 14. In another study carried out on 100 adults of both sexes in whom the possibility of hepatic and neoplastic disease had been excluded, the average portal pressure was found to be 215 mm water and ranged from 100 to 300 mm.<sup>130</sup>

Causes of portal hypertension have been mentioned (page 1414). It is recognized that

the liver may be diseased long before significant clinical evidence is found. A high incidence of hepatic abnormality (68% or more) has been reported in patients with Banti's syndrome.<sup>104,122,146</sup> Noteworthy also is the fact that splenomegaly of some degree has been found in as many as 79% of persons with cirrhosis.<sup>81</sup>

The hematologic changes in patients with Banti's syndrome are attributable to the unequal distribution of the cells of the blood in the splenic vascular bed and the remaining parts of the circulation.<sup>138</sup> This is the consequence of stasis of the portal circulation. In addition, there is increased destruction of blood cells, as will be discussed in the following section of this chapter ("hypersplenism"). The rate of removal of damaged red cells in the spleen has been found to correlate best with spleen size and poorly with portal pressure.<sup>117</sup>

No clinical or hematologic differences between patients with congestive splenomegaly due to intra- or extrahepatic obstruction have been observed, except in those with advanced liver disease. Furthermore, the histopathology of the spleen has been the same in all the patients, no matter what the cause of the portal hypertension happened to be. Following splenectomy, blood values usually have returned to normal. These observations strongly support the view that Banti's syndrome is the consequence of any of a variety of lesions producing chronic splenic vein hypertension.

Partial or complete occlusion of the portal vein<sup>129</sup> in rats and rabbits failed to produce the typical Banti syndrome. However, when hepatic cirrhosis was produced in rabbits by manganese<sup>127</sup> or in dogs by means of silica,<sup>136,141</sup> splenic enlargement and the picture of congestive splenomegaly did develop. Congestive splenomegaly also has been produced by the intraperitoneal administration of methyl cellulose<sup>112,133</sup> (page 1418).

Failure to demonstrate an obstructive factor in a number of cases and the fact that portal obstruction independent of splenic disease has been observed in the absence of appreciable splenic enlargement led to an

alternative concept<sup>136</sup> which, while accepting the significance of splenic congestion, revived the theory of "primary" splenic disease. It was suggested that the small splenic arteries play an important role in adjusting the intake of blood into the spleen. It was argued that, because of disease of these arteries, blood enters the spleen in increased quantity, leading to congestion, and this in turn causes portal hypertension and even degenerative changes in the liver cells.

## The Concept of "Hypersplenism"

### History and Definition

The concept that the "organ of mystery," as Galen called the spleen, can be a cause of disease seems plausible since this organ, which is normally not palpable, may become enlarged under certain circumstances, and then anemia, leukopenia, or thrombocytopenia or combinations thereof are not uncommonly associated. In such patients, these clinical manifestations may disappear following splenectomy.

The idea that the spleen may produce ill effects through exaggeration of its normal activities was entertained in 1866 by Gretscl and in the 1880's by Banti. In 1907, Chauffard introduced the term "hypersplenism" to refer to this concept.<sup>155,160,173</sup> However, this hypothesis did not become popular until many years later.<sup>168,169</sup> and since then there has been considerable confusion concerning the nature or meaning of the term and the pathogenesis of such a condition.<sup>162</sup> Criteria for a diagnosis of "hypersplenism" include: (1) anemia, leukopenia, thrombocytopenia, or combinations thereof; (2) cellular bone marrow; (3) splenomegaly; and (4) improvement following splenectomy.<sup>155</sup>

### Classification

"Hypersplenism" has been classified as "primary" and "secondary."<sup>155,166,173,200</sup> The

term "secondary hypersplenism" has been used in referring to cases in which some more or less well-defined disorder can be identified,<sup>155,163,166,180</sup> while "primary hypersplenism" refers to those in which an underlying disease cannot be found.<sup>155,165</sup> Thus, "secondary hypersplenism" includes a variety of infectious<sup>163</sup> or parasitic disorders<sup>190</sup> and certain storage diseases, such as Gaucher's disease, when they involve the spleen, as well as some cases of leukemia and lymphosarcoma<sup>192</sup> in which cytopenia is prominent and is relieved by splenectomy. Congestive splenomegaly (page 1412) and Felty's syndrome are also included. "Hypersplenic" syndromes have even been described in association with hyperthyroidism<sup>179</sup> and with urticaria pigmentosa.<sup>175</sup> Some have included hereditary spherocytosis, other hemolytic anemias, and idiopathic thrombocytopenic purpura as forms of hypersplenism.<sup>155,168,173</sup> Primary hypersplenic syndromes include "primary splenic neutropenia," "primary splenic panhematopenia," and "splenic anemia," discussed on page 1410.

It is true that many or all of these conditions fulfill the criteria for the diagnosis of hypersplenism listed above. Whether the use of this designation is helpful is less obvious.

### Pathogenesis

The functions of the spleen are discussed in Chapter 8. Although much still remains to be learned about this organ, it has been clearly established<sup>190</sup> that (1) an enlarged spleen may sequester large numbers of red cells, platelets, and granulocytes, which (2) may be damaged there or be destroyed; and (3) total plasma volume may increase in association with splenomegaly, resulting in some degree of "pseudo-anemia." There is, on the other hand, little evidence that humoral factors are produced in the spleen as some investigators<sup>162,194</sup> have proposed.

The pathogenesis of the hematologic changes that may be associated with big spleens has been studied with radioactive isotopic labeling techniques. Variable degrees of splenic sequestration and/or increased cell

destruction have been found.<sup>156,158,161,165,172,190</sup> Furthermore, it has been demonstrated that in patients with massive splenomegaly from a variety of causes (including nontropical splenomegaly,<sup>165</sup> tropical splenomegaly,<sup>190</sup> cryptogenic splenomegaly,<sup>76</sup> congestive splenomegaly,<sup>161,172</sup> myelofibrosis,<sup>161,172,177,181</sup> chronic lymphocytic leukemia,<sup>181</sup> and chronic myelocytic leukemia,<sup>172,181</sup> as well as other causes<sup>161,172</sup>), an apparent *anemia*, as judged by venous hemoglobin or packed red cell concentration, is often due to expansion of the plasma volume in the presence of a normal red cell mass, a significant portion of which is sequestered in the enlarged spleen.<sup>190</sup> Expansion of the plasma volume in the presence of a normal red cell mass may be detected by simultaneously measuring the red cell mass and plasma volume and by comparing the total body to venous hematocrit ratio. The latter has been found to be above the normal range of  $0.896 \pm 0.039$ <sup>177</sup> in most patients with enlarged spleens. It was demonstrated that the ratio increased progressively in direct proportion to the degree of splenic enlargement.<sup>177</sup> As much as 38% of the total red cell mass may be trapped in a massively enlarged spleen.<sup>190</sup> Reduction in red cell survival time may or may not be found,<sup>43,172,190</sup> but the anemia appeared to be due mainly to hemodilution and sequestration.<sup>161,172,181</sup> In this regard, it should be noted that hypervolemia with an expanded plasma volume and normal or only slightly reduced red cell mass plus moderate anemia has been found in patients with cirrhosis even when no striking splenomegaly was present.<sup>186</sup> It is postulated that the plasma volume in the expanded portal vascular space probably is increased. This may be the result of a marked increase in metabolism and a decrease in peripheral resistance leading to salt and water retention through the renin-angiotensin aldosterone mechanism.<sup>181</sup>

*Leukopenia and neutropenia* in association with splenomegaly appear to be due to increased destruction in some cases<sup>158</sup>; in others, sequestration may possibly play a role. Again, in association with splenomegaly as much as 50 to 90% of the total *platelet*

*mass* may be sequestered in an enlarged spleen.<sup>156</sup>

Animal experiments support the concept that abnormal blood cell sequestration and destruction occur in a hyperplastic spleen. Thus, in rats injected with methylcellulose to produce splenomegaly and anemia, transfused normal rat erythrocytes were sequestered and destroyed in the enlarged spleen.<sup>112,188</sup> In contrast, red cells from the methylcellulose-injected rats survived normally when transfused into normal animals. Thus, methylcellulose-injected rats suffered from an extracorporeal hemolytic anemia that required the presence of a hyperplastic spleen.<sup>149</sup> It is of interest that suckling offspring of hypersplenic animals also became anemic, thus suggesting that hormonal factors may have been operative.<sup>162</sup>

As understanding has grown, conditions that at one time were attributed to "hypersplenism" have been shown to be due primarily to defects in tissues other than the spleen. Thus the fault in hereditary spherocytosis rests in the red corpuscle itself. Likewise "idiopathic" thrombocytopenic purpura is attributable in most instances to antibodies and the fault does not rest primarily in the spleen. Even though the defective or damaged blood elements are sequestered and/or destroyed in the spleen and splenectomy alleviates the manifestations, application of the term "hypersplenism" to these conditions is misleading since the primary defect is not in the spleen. In regard to other conditions characterized by anemia, leukopenia, and/or thrombocytopenia in association with splenomegaly and whose cause is as yet not understood, such as "primary splenic neutropenia" and "primary splenic panhematopenia," it is reasonable to expect that in most, and perhaps all, instances the primary fault will be found in sites other than the spleen, with the spleen playing a secondary role. In this sense the term "hypersplenism" tends to hide our limited knowledge by implying a pathogenic mechanism that is imprecise and probably erroneous. Therefore, we prefer to avoid its use and wherever possible we refer specifically to such mechanisms as sequestration or destruction, or other mechanisms by which

the observed hematologic changes may be produced.

## Methods for Evaluating Suspected Splenomegaly

A palpable mass in the left upper quadrant of the abdomen, although often an enlarged spleen, may be due to other causes such as gastric, pancreatic, ovarian, or renal cysts or tumors. Appropriate studies including an intravenous pycnogram and barium swallow will usually suffice to rule out the possibility of renal and gastric pathologic conditions. The possibility of pancreatic cysts or tumors may be more difficult to exclude with conventional radiographic techniques.<sup>85</sup>

### Splenoportography

The presence of pancreatic cysts has occasionally been revealed when splenoportography was carried out in an attempt to visualize what was thought to be a large spleen.<sup>206</sup> However, splenoportography has found wide use chiefly in evaluating: (1) the patency of the portal vein and the distribution of collateral circulation prior to shunt operations for cirrhosis; (2) the cause of esophageal varices in the absence of liver disease; and (3) the cause of idiopathic splenomegaly (especially in children).<sup>100,111,208</sup> It probably should be avoided if the platelet concentration is low or if there is an increased tendency to bleed since 50 ml of blood or more are regularly found in the abdomen after this procedure<sup>208</sup> even when clotting function is normal.

### Angiography

Selective angiography is probably a safer and more useful procedure than splenoportography for differentiating splenic cysts or other abnormalities in the spleen from nonsplenic tumors.<sup>216,229</sup>

### Spleen Scans

Enlargement of the spleen as assessed subjectively by the radiologist viewing an ordi-

nary film of the abdomen is less accurate in predicting splenomegaly than is definite palpability.<sup>4</sup> Likewise, ordinary abdominal films are often unsatisfactory in that the spleen is not visualized in as many as 40% of those examined.<sup>4</sup> However, nonpalpable spleens may be enlarged, and it has been claimed that in only 30% of patients with enlarged spleens as determined by scans has the organ been palpable.<sup>222</sup>

The best noninvasive method for evaluating spleen size is the spleen scan. In 17 patients scanned 24 to 72 hours before splenectomy a close correlation between spleen length on scan and spleen weight after surgical removal was demonstrable ( $r, +0.96$ ).<sup>219</sup> In another study, spleen mass as estimated from scans was compared with spleen weight at postmortem examination. Errors of less than 12% (mean of 7%) were found over a wide range of spleen sizes (weights, 180 to 2000 g).<sup>223</sup>

Several techniques are available for spleen scanning; these include labeling erythrocytes with <sup>51</sup>Cr, <sup>197</sup>Hg, <sup>81</sup>Rb, or <sup>99m</sup>Tc<sup>210,221</sup> and mildly damaging the cells by means of treatment with heat, antibody, chemicals, or metal ions so that they will be sequestered by the spleen after infusion. More generally used are colloidal suspensions of radionuclides such as technetium (<sup>99m</sup>Tc), gold (<sup>198</sup>Au), or indium (<sup>113m</sup>In).<sup>221</sup> The relative merits of these techniques are discussed in several reviews.<sup>221</sup> In addition to assessing spleen size, spleen scans may be useful in detecting space-occupying lesions in the splenic substance<sup>221</sup> (Fig. 45-2), and in evaluating loss of spleen function, absence of the spleen, or the presence of an accessory spleen.<sup>221</sup>

### <sup>51</sup>Cr-Labeled Red Cells

Most widely used in evaluating the degree of splenic sequestration and/or cell destruction in the spleen of patients with anemia are data obtained after the infusion of <sup>51</sup>Cr-labeled red cells. The subsequent measurement of red cell mass (Chapter 3), red cell survival in the blood (Chapter 5), the rate of mixing in the spleen, and localization of label in the spleen as compared with the liver and precordium

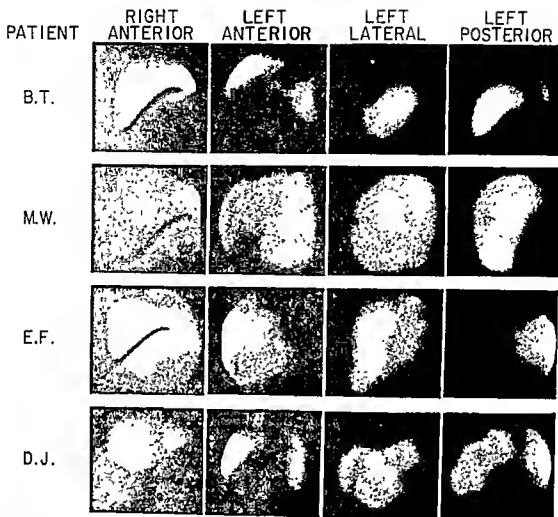


Fig. 45.2 Illustrative <sup>99m</sup>Tc sulfur colloid liver and spleen scans.

*Patient B.T.* had no disease involving the liver or spleen. Note the size and position of the normal liver and spleen in the four panels. The diagonal shadow in panel one is produced by a lead marker placed along the costal margin.

*Patient M.W.* had acute myelomonoblastic leukemia and *E. coli* septicemia at the time of the scan. Note the large liver and spleen in the first two panels and the filling defects in the spleen, more apparent on the left lateral and left posterior views. Splenectomy revealed several splenic abscesses containing *E. coli*.

*Patient E.F.* Polycythemia vera. The spleen was palpable, but numerous Howell-Jolly bodies were present in the blood smear. The liver was large (panel one). The left lobe extended into the left upper quadrant (panel two) and was visible in the anterior portion of the left lateral view. However, the spleen was not visualized. It is presumed that thrombosis of the splenic artery produced functional asplenia.

*Patient D.J.* Liver and spleen scan in a patient who suffered abdominal injuries in an accident. The liver appears intact but several defects are visible in the upper and lower poles of the left lateral and left posterior views. These findings were confirmed by arteriography and several hematomas were found at surgery.

(Prepared with the assistance of Dr. Harold DeBlanc, Division of Nuclear Medicine, University of Utah College of Medicine.)

(Chapter 27) provides useful information.<sup>221,223</sup>

The *total body to venous blood hematocrit ratio* (TB/VH) has been used to assess the degree of *red cell pooling* in enlarged spleens, and was discussed on page 1418.

*Splenic mixing curves* have also proved useful in assessing red cell pooling. These are obtained by continuous counting over the spleen during the first few minutes to an hour after injection of <sup>51</sup>Cr-labeled red cells. If the counts are plotted as the logarithm of the



equilibrium value ( $A_x$ ) minus the value at any time  $t$ , ( $A_t$ ), evaluation of the data is simplified (Figs. 45-3 and 45-4). In normal man the mixing curve consists of a single exponential (Fig. 8-3, page 358), which reaches equilibrium within 1.5 minutes ( $0.91 \pm 0.19$  minute).<sup>228</sup> This curve differs little from that obtained over the heart and reflects the rapid mixing of labeled cells in the normally small (20 to 30 ml) splenic red cell pool.<sup>213,224</sup>

A single-component curve but with a longer than normal  $t_{1/2}$  may be obtained in some patients with splenomegaly (Fig. 45-3).<sup>213</sup> This occurs in congestive splenomegaly and reflects slower than normal mixing in a large pool that is located mainly in the splenic sinusoids.<sup>224</sup> Single-component mixing curves are not associated with splenic hemolysis.<sup>213</sup>

#### Two-component splenic mixing curves

(Fig. 45-4) are obtained if abnormal cells (eg, from patients with hereditary spherocytosis) are transfused into a normal recipient<sup>213</sup>; they also are found in some patients with splenomegaly even after transfusion with normal cells.<sup>213</sup> Two-component mixing curves are the usual kind obtained in patients with hereditary spherocytosis, autoimmune hemolytic anemia, and in some with lymphoma or other forms of splenomegaly.<sup>224</sup> The faster component (line A in Fig. 45-4) reflects slow mixing in the expanded splenic sinusoidal pool, while the slower component (line B in Fig. 45-4) reflects cell trapping (pooling) in the red pulp.<sup>224</sup> Two-component mixing curves may or may not be associated with splenic hemolysis,<sup>213</sup> but some increase in red cell destruction occurs in most enlarged spleens.<sup>223</sup>

When measurements of radioactivity in the

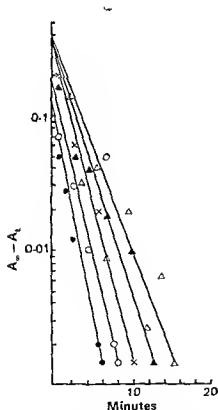


Fig 45-3. Single-component exponential curves depicting the time course of counting rates over the spleen after the injection of  $^{51}\text{Cr}$  labeled red cells into five subjects with splenomegaly. Ordinate (log scale) =  $A_x - A_t$ ; abscissa = time in minutes (From Harris, McAllister, and Prankard,<sup>213</sup> courtesy of the authors and the British Journal of Haematology)

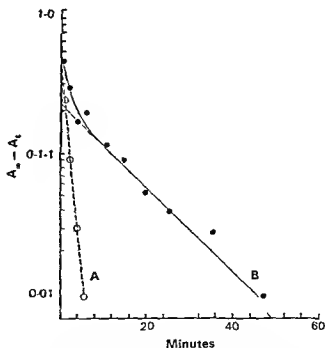


Fig 45-4. Two-component curve (solid line) depicting the counting rates over the spleen of a patient with idiopathic splenomegaly given an injection of  $^{51}\text{Cr}$ -labeled red cells

Ordinate (log scale) =  $A_x - A_t$ ; abscissa = time in minutes. Interrupted line = rapid exponential obtained by plotting differences between points on curve B and the values on extrapolation to zero time (From Harris, McAllister, and Prankard,<sup>213</sup> courtesy of the authors and the British Journal of Haematology)

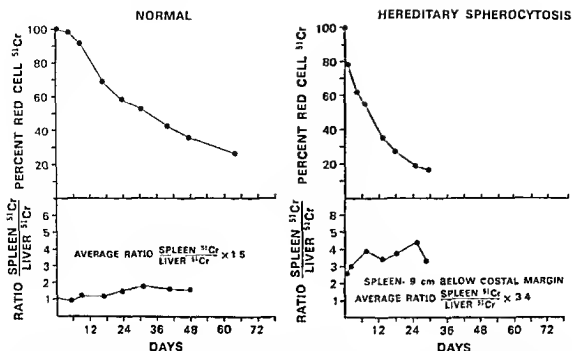


Fig 45-5 Red cell survival and spleen-to-liver ratio curves obtained after the infusion of washed autologous erythrocytes labeled with  $^{51}\text{Cr}$ . Note the shortened red cell survival and increased spleen-to-liver ratio in the patient with hereditary spherocytosis as compared to the normal subject (From Schlosser, Korst, Clatanoff and Schilling,<sup>227</sup> courtesy of the authors and the Journal of Clinical Investigation)

blood and over various organs (*in vivo*  $^{51}\text{Cr}$  localization) are made at intervals extending over a few days to several weeks, still more useful data are obtained.<sup>215,227</sup> Curves depicting the relative accumulation of label in the spleen and liver as compared with the precordium<sup>215</sup> or the spleen-to-liver count ratios<sup>227</sup> may provide evidence of predominant accumulation in one or the other organ (Fig 45-5). Although different indices and modes of data presentation have been used<sup>212,215,217,227</sup> there is general agreement that benefit from splenectomy can be predicted if there is shortening of the red cell  $t_{1/2}$  to less than 15 days and associated progressive accumulation of radioactivity over the spleen. In this regard, a high spleen-to-liver ratio (greater than 2.3) at  $t_{1/2}$  appears to be one of the most useful prognostic indicators.<sup>205,212</sup> Nevertheless, occasional patients with lesser degrees of splenic sequestration have shown an excellent response to splenectomy.<sup>205</sup> This subject has been discussed in Chapter 27 (page 915).

### $^{51}\text{Cr}$ -Labeled Platelets

Similar studies of recovery in the blood after infusion of  $^{51}\text{Cr}$ -labeled platelets and the accumulation of these platelets in the spleen have been utilized to evaluate patients with splenomegaly.<sup>156</sup> This subject has been discussed in Chapter 34. Platelet labeling is less useful in predicting the outcome of splenectomy than are red cell studies.<sup>217</sup>

### Splenic Puncture

American hematologists were slow in adopting biopsy procedures. This was true for bone marrow examination and is still true for splenic and lymph node punctures. Splenic puncture was employed as a means of obtaining material for bacterial culture by Widal and his school at the turn of the century and has been used as an aid in the study of splenomegalies in Europe and in Asia since that time.<sup>238</sup> Lymph node examinations have also been practiced by Europeans for a number of years.<sup>231,233</sup>

Moeschlin<sup>238</sup> recommends that splenic puncture be carried out only when there is distinct splenic enlargement and that it be performed with strictest aseptic technique. The procedure is contraindicated in patients with hemorrhagic manifestations, in those with painful splenomegaly (infarct, etc.) or enlargement due to acute sepsis, and in those who are lethargic or cannot cooperate.

#### *Technique*<sup>238</sup>

The spleen should be carefully outlined. If it is not palpably enlarged, it must be outlined by means of percussion. The patient should also be examined fluoroscopically. By giving a carbonated beverage or 10 g of bicarbonate in a little water followed by a small amount of lemon juice, a bubble of gas is produced in the stomach; the fluoroscope will then show the spleen lying between the gastric gas bubble and the diaphragm.

The puncture is performed in the ninth or tenth intercostal space in the midaxillary line during complete inspiration (Fig. 45-6). Complete inspiration is strongly recommended because a reflex inspiratory descent of the diaphragm is sometimes produced as the needle enters the abdominal cavity and, if the puncture is being done during expiration, there is risk of lacerating the spleen. Puncture of adjacent organs is avoided by using the site recommended. Puncture through the abdominal wall is not desirable as this, even under the best of circumstances, runs the risk of puncture of viscera or displacement of the needle by contraction of the abdominal muscles.

The patient lies flat on his back without pillows under his head. The splenic area is outlined and the lowest limit of pulmonary resonance during full inspiration is determined by light percussion. A site 5 cm below this level is marked. This is where the needle

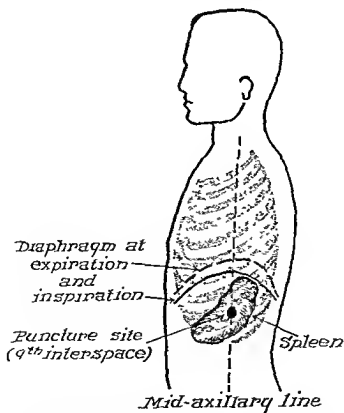


Fig. 45-6 Splenic puncture. The preferred site of puncture is indicated (From Ferris and Hargraves,<sup>236</sup> courtesy of the authors and Archives of Surgery)

will be inserted. The skin is then painted with an appropriate antiseptic and draped with a sterile eyecloth.

For anesthesia, 4 ml of 2% procaine solution in a 5-ml record syringe, to which is attached a fine needle, 10 cm in length, are used. A cutaneous wheal is produced and then the underlying tissues are infiltrated perpendicularly to the skin surface to a depth of 1 to 2 cm. The patient is asked to breathe rapidly but superficially while the needle is slowly pushed more and more deeply, 1 mm at a time, until slight pain is produced. The development of pain indicates that the peritoneum has been penetrated. The needle is finally pushed 1 to 2 mm further until a scratching sensation is felt as it rubs against the spleen. The depth from the skin to this point is marked on the needle.

For splenic puncture a needle 12 to 15 cm long and 1.2 to 2.0 mm in diameter is recommended. There should be a ground-in stylet and the bevel must not be steep but it should be extremely sharp. A movable guard should be attached to the needle. A 20-ml record syringe is preferred to a smaller one since this permits stronger suction. The needle and syringe must be dry.<sup>236</sup>

On the splenic puncture needle, a distance is marked by setting the guard in a position that is equal to the depth marked on the anesthesia needle plus 2 cm. The additional 2 cm make it possible to penetrate the spleen to a depth of 2 cm and no more. This prevents too deep penetration of the spleen and avoids damage to the larger vessels of that organ. The larger vessels enter at the hilum and end in a fine meshwork at the subcapsular region.

In performing the puncture, the needle is pushed through the spot marked on the skin, the stylet is then removed and the syringe is attached. The patient is told to take a deep breath, close his mouth, and pinch his nose. The needle is then pushed in rapidly to the hilt of the guard. Aspiration is carried out quickly but strongly one or two times. The needle is then withdrawn, but before doing this, negative pressure is gradually released by permitting the plunger to come back

slowly. If this is not done, the negative pressure will bring blood or the anesthetic agent into the syringe and ruin the preparations.

It is important that the patient hold his breath throughout the whole procedure. Before performing the puncture, it is necessary to have the patient practice his role in the steps required. Following the procedure, the patient must remain in bed, lying flat on his back for one hour. At the end of this time he may be given a meal but is asked to remain in bed for another six hours.

Moeschlin has successfully performed many hundred splenic punctures by this technique, without accident. Others<sup>239,242</sup> have used, without trouble, a 20-gauge needle to aspirate, even intercostally, in the area of maximal dullness when the spleen has not been palpably enlarged. With such a needle it was not found necessary to employ a preliminary local anesthetic.

Biopsy with the Vim-Silverman needle is more dangerous and several deaths have occurred following the procedure.<sup>242</sup> Sections obtained with such a needle are especially useful in disorders such as amyloidosis or sarcoidosis in which architecture is more important than individual cell structure.

### *Indications and Interpretation*

The material obtained by splenic puncture usually consists of 1 to 3 drops of rather bloody fluid and a few fragments of tissue. On microscopic examination, 60 to 90% of the cells are found to be lymphocytes. The remainder include granulocytes and reticuloendothelial cells. The latter are mainly derived from the sinuses. Moeschlin's "pulp cells" (Fig. 45-7) have not been observed in tissue obtained from gland or marrow punctures. Other important elements include typical plasmacytoid reticulum cells, identical with those of the marrow, and large lymphatic plasma cells of the same type as those obtained in gland punctures.

Although differential counts (splenograms) can be carried out, an exact splenogram is not usually necessary. Careful examination of films will give a sufficiently accurate idea of

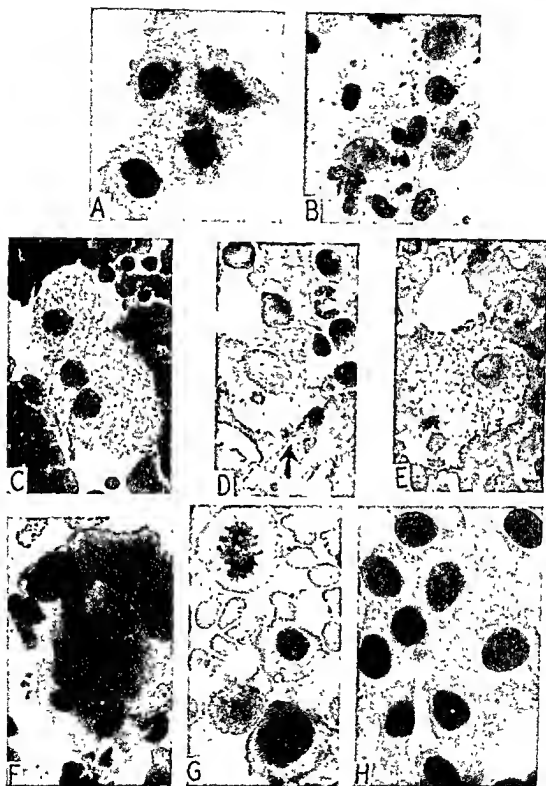


Fig. 45-7. Cells obtained by splenic puncture (Courtesy of Dr. S. Moeschlin) A, Splenic pulp cells, B Dorothy Reed cells in Hodgkin's disease, C, Gaucher cells, D, spleen macrophage with vacuolated cytoplasm, E, Niemann-Pick cell, F, multinucleated epithelioid cell in tuberculosis, G, megaloblasts, pernicious anemia H, hypernephroma cells, from tumor mistaken for spleen

the distribution of the cells and will reveal the presence of abnormal ones.

Splenic puncture<sup>211</sup> is useful in the diagnosis of disorders due to parasites, such as leishmaniasis and malaria; it may serve to differentiate one of the fat-storage disorders from atypical leukemia or splenic neutropenia, and it may be helpful in distinguishing leukemoid reactions from true leukemia.<sup>233,241,242</sup> Splenic puncture also makes possible the demonstration of Dorothy Reed cells in the spleen in patients with abdominal Hodgkin's disease and of epithelioid cells in patients with tuberculosis and in those with brucellosis<sup>219</sup> (Fig. 45-7). In other conditions, the procedure has less importance. In the presence of cirrhosis of the liver and of splenoportal thrombosis, the splenogram is practically normal, even though the spleen may be greatly enlarged. In these conditions the puncture specimen is likely to be unusually bloody. In hemolytic anemia a fairly normal picture has been observed although, as the result of increased blood destruction, hemosiderin-containing macrophages may be quite numerous and some erythroblasts may be observed during periods of exacerbation. Moeschlin did not find signs of phagocytosis of red corpuscles. In patients with pernicious anemia, megaloblasts are plentiful in the spleen.

Hemopoietic foci in the spleen have been described in association with inflammatory splenomegalies.<sup>238</sup> In acute inflammatory reactions, lymphocytes are reduced in number while neutrophils, including stab cells and myelocytes, are increased as are also the pulp cells and macrophages. Only an occasional erythroblast is seen. In chronic inflammatory splenomegalies, in addition to these changes, monocytes, plasmacytoid reticulum cells, and sometimes erythroblasts are increased in number. Cells from these foci may enter the blood in sufficient numbers to arouse a suspicion of early chronic myelocytic leukemia when splenomegaly and leukocytosis are present. In patients with leukemia, however, young myeloid cells form 20 to 60% of the cells whereas in those with chronic inflammatory splenomegaly they do not rise above

1 to 5%. In patients with myelofibrosis with myeloid metaplasia, the persistence of large numbers of lymphocytes (50 to 60%) distinguishes the condition from myelocytic leukemia.<sup>235</sup>

Lymphocytic leukemias are characterized by an almost purely lymphocytic picture (92 to 99%). The cells often vary greatly in size and lymphoblasts in mitosis may be prominent. Normally, mitoses in lymphocytes are rare, their mitotic index having been found to be 50 times less than that of granulocytes. In patients with lymphoid leukemia, the mitoses may be increased 15 or 30 times the normal, although this is not found in all these subjects.

In infectious mononucleosis, at the height of the disease a considerable excess of reticuloendothelial cells, some in mitosis, with transitions between them and the typical infectious mononucleosis cells has been described. In infectious hepatitis, many of the immature precursors of the lymphatic plasma cells that are found in the blood have been observed. Even though splenic aspiration may be diagnostic in from 15 to 33% of patients with splenomegaly<sup>211,212</sup> it has not become a popular procedure in many parts of the world. This may be because it only provides confirmatory information in about one third of the patients studied, is nondiagnostic in an additional one third, and may be misleading or confusing in 10 to 15%.<sup>211,242</sup>

## Indications for Splenectomy

Even though our understanding of splenic function is incomplete, clinical experience affords a good foundation upon which a decision for or against splenectomy in a given patient can be made. Such experience can also be fitted reasonably well with what is known concerning functions of the spleen, as discussed in Chapter 8. Thus, in general, splenectomy has its greatest value when the spleen appears to be exerting a destructive effect on blood cells and is usually unnecessary and unwise when it is serving a con-

structive role, such as antibody production or hematopoiesis.

Vague references to removal of the spleen have been found in ancient Greek and Roman literature extant before the birth of Christ.<sup>249</sup> However, splenectomy in man was apparently first clearly recorded in 1549 and experimental splenectomy in dogs was performed in 1680. Therapeutic splenectomies were recorded in 1826, 1855, and 1865, but the patients all died.<sup>247</sup> Nevertheless, by the late 1800's, series of splenectomies were being reported, chiefly for treatment of cysts, abscesses, injuries, and wounds and for removal of massively enlarged spleens. In one such series reported in 1898, 62% of the patients recovered.<sup>247</sup> As a result of this relative success, splenectomy then was tried in a variety of hematologic diseases with variable results. By 1930 in two series, one of 500 and the other of 118 patients, operative mortality when splenectomy was performed because of thrombocytopenic purpura or congenital hemolytic jaundice had decreased to less than 5%.<sup>254,256,258</sup> However, the results of splenectomy for cirrhosis, sepsis, leukemia, pernicious anemia, and aplastic anemia were less good and operative mortality was higher.

Indications for splenectomy have been discussed in relation to specific disorders in various chapters in this book. There now is general agreement that splenectomy is: (1) regularly beneficial in hereditary spherocytosis (Chapter 21); (2) often useful in idiopathic thrombocytopenic purpura (Chapter 34); (3) beneficial in many patients with acquired hemolytic disease (about 50%) (Chapter 27); and (4) may be beneficial in carefully selected patients with congestive splenomegaly with associated cytopenia.<sup>246,249,260</sup> To these indications some have added (5) Hodgkin's disease, and perhaps some cases of non-Hodgkin's lymphoma for the purpose of staging prior to initiation of therapy (Chapters 50 and 51). Splenectomy in patients with other conditions is more controversial but is indicated under special circumstances. Thus, indications for splenectomy in thalassemia (Chapter 26), the hemoglobinopathies (Chapters 24 and 25), nonspherocytic hemo-

lytic anemias (Chapters 20, 21, and 22), aplastic anemia and pancytopenia (Chapter 56), chronic myelocytic leukemia (Chapter 48), myelofibrosis (Chapter 57), polycythemia vera (Chapter 30), and Gaucher's disease (Chapter 42) are discussed in the appropriate chapters. As stated earlier in this chapter (page 1410), in certain patients with splenomegaly associated with neutropenia or pancytopenia of obscure cause, splenectomy has been claimed to be helpful.<sup>170,250,255</sup>

The necessity for splenectomy in patients having traumatic splenic rupture is obvious. In several centers, 25% of all spleens removed were taken out because of iatrogenic trauma incidental to other abdominal operations.<sup>259</sup> Removal of the spleen because of large cysts or tumors, whether primary or secondary, is relatively unusual.<sup>253</sup> When the cause of splenomegaly has not been clear, and careful evaluation has been inconclusive, *diagnostic laparotomy and splenectomy* revealed lymphoma in 31%, congestive splenomegaly in 25%, and inflammatory disease in 19% of a series of 52 patients reported by Hermann and associates.<sup>251</sup> Similar findings have been reported by others.<sup>245</sup>

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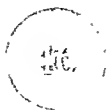
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### SECTION 3: *Neoplastic Diseases of the Hematopoietic System*

The leukemias and lymphomas, as well as the myelomas and other immunoglobulin-secreting tumors, are considered in this section. The clinical patterns and treatment of each major disease or group of diseases will be reviewed in separate chapters (Chapters 47-53); their classification and possible etiologic basis and their pathogenesis will be considered in Chapter 46. These diseases have many complications in common, but certain complications are more specifically related to certain diseases than to others. The complications of the individual diseases will be described in the chapters dealing with each specific disease; the nature and treatment of the various complications in general will be considered in a special chapter (Chapter 54). Specific methods of treatment for each disease are given in the respective chapters. A detailed discussion of the nature and use of currently available drugs is presented in a separate chapter (Chapter 55).

The cause of neoplastic diseases of the hematopoietic system is unknown. All are characterized by abnormalities in the growth of hematopoietic cells. In certain instances the diseases are curable, but usually they are not, although therapy will ameliorate symptoms and in some patients it will prolong life.

The diagnosis of the leukemias, lymphomas, myelomas, and related diseases is established by demonstrating an increased, and often continually increasing, number of specific hematopoietic cells in the absence of a demonstrable, appropriate stimulus. Cellular excess may be localized to a single lymph node, as in some patients with Hodgkin's disease, or may be quite widespread and involve bone marrow, blood, and many lymph nodes as well as other organs, as occurs in most patients with leukemia. Certain characteristic signs, symptoms, and laboratory findings are anticipated in each disease and these serve as corroborative diagnostic evidence. In certain diseases it is clear that the excessive number of cells is the result of excessive cell proliferation, while, in others, increased life span of the cells may be a primary factor in the cellular accumulation.



# *Classification, Pathogenesis, and Etiology of Neoplastic Diseases of the Hematopoietic System*

Classification and Its Historical Development
Interrelation of the Diseases
Incidence
Pathogenesis
Evidence for Clonal Disease
Chromosome Abnormalities in Tumor Cells
Functional and Biochemical Abnormalities of Tumor Cells
Cell Kinetics
Control Mechanisms
Etiology
Viruses
Other Infecting Agents
"Clustering" of Patients with Leukemia, Lymphoma, and Myeloma
Other Environmental Factors
Irradiation
Chemical Agents
Familial Disease
Ethnic Differences
Increased Frequency of Leukemia in Diseases Associated with Congenital Chromosomal Abnormalities
Association with Other Diseases

## **Classification and Its Historical Development**

The disease classification presented in Table 46-1 is derived from the fact that the major disease categories differ with respect

to morphologic and clinical manifestations, and often in their response to therapy. Few authorities would disagree concerning the recognition of chronic myelocytic leukemia (CML), acute myeloblastic leukemia (AML), chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), Hodgkin's disease (HD), the non-Hodgkin's lymphomas (NHL), multiple myeloma (MM), and macroglobulinemia as distinct entities. Subdivisions of these categories have been proposed, as discussed below and as shown in Table 46-1.

Classification of these diseases has developed out of their gradual historical recognition and the differences observed between them. Thus, in 1666, Malpighi described a fatal disease in which the lymphoid tissue and the spleen appeared "like a cluster of grapes."<sup>7</sup> In 1832, Hodgkin<sup>13</sup> described the gross anatomy of seven patients with fatal illnesses associated with lymph node enlargement; the disease in three of these patients would now be assigned the eponym to which Hodgkin's name is given.<sup>5</sup> It was Wilks, however, who, in 1856, provided a lucid description of HD as it is now recognized.<sup>7</sup> The studies of Kundrat<sup>14</sup> (1893), Longcope<sup>16</sup> (1907), and others led to the clear pathologic separation of HD from NHL. Roulet<sup>24</sup> distinguished reticulum cell sarcoma from other

**Table 46-1. Classification of the Neoplastic Diseases of the Hematopoietic System**

- I **Leukemias**
  - A Granulocytic leukemias
    - 1 Chronic myelocytic leukemia (myelogenous granulocytic)
      - a Atypical chronic myelocytic leukemia
      - b Chronic eosinophilic leukemia
      - c Chronic basophilic leukemia
      - d Chronic monocytic leukemia
    - 2 Acute myeloblastic leukemia (myelocytic, granulocytic, myelogenous)
      - a Acute myelomonoblastic leukemia
      - b Acute monocytic leukemia
      - c Acute promyelocytic leukemia
      - d Acute erythroblastic leukemia
      - e Acute eosinophilic leukemia
      - f Acute basophilic leukemia
      - g Chloroma
  - B Lymphocytic leukemias (lymphoid lymphogenous)
    - 1 Chronic lymphocytic leukemia
      - a Chronic lymphosarcoma cell leukemia
      - b Sezary syndrome
    - 2 Acute lymphoblastic leukemia (stem cell)
      - a Acute lymphosarcoma cell leukemia
- II **Lymphomas (primary tumors of lymphoid organs)**
  - A Hodgkin's disease
    - 1 Nodular sclerosing
    - 2 Lymphocyte predominance
    - 3 Mixed cellularity
    - 4 Lymphocyte depletion
  - B Non Hodgkin's lymphomas
    - 1 Lymphocytic lymphomas (lymphosarcoma)
      - a Well-differentiated lymphocytic (nodular or diffuse)
      - b Poorly differentiated lymphocytic (nodular or diffuse)
    - 2 Histiocytic lymphomas (reticulum cell sarcoma), nodular or diffuse
    - 3 Mixed histiocytic lymphocytic lymphomas, nodular or diffuse
    - 4 Mycosis fungoides
    - 5 Burkitt's lymphoma
- III **Unusual tumors possibly related to granulocytic leukemias and lymphomas**
  - A Mast cell leukemia
  - B Histiocytic medullary reticulosis
  - C Reticuloendothelioses
- IV **Immunoglobulin secreting tumors**
  - A Myeloma
    - 1 IgG proteinemia
    - 2 IgA proteinemia
    - 3 IgD proteinemia
    - 4 IgE proteinemia
    - 5 Light-chain proteinuria
  - B Macroglobulinemia
  - C Heavy-chain disease

lymphosarcomas; giant follicle lymphoma was first described by Ghon and Roman<sup>10</sup> and was recognized as clinically distinctive by Brill et al<sup>3</sup> as well as by Symmers.<sup>27</sup> The giant polyploid macrophage which most consider the *sine qua non* of HD was first described by Langhans,<sup>15</sup> again by Greenfield,<sup>12</sup> and later by Sternberg, who mistakenly attributed it to tuberculosis.<sup>25</sup> Reed<sup>21</sup> was the first to emphasize its unique relationship to HD and the eponym "Reed-Sternberg" cell is commonly applied.

Leukemia was distinguished as a separate clinical entity by Craigie, Bennett,<sup>1</sup> and Virchow<sup>29</sup> in 1845. Virchow recognized that the cells involved did not represent suppuration of the blood and proposed the name "leukemia" (white blood). He later distinguished two forms of leukemia, one in which splenomegaly predominates and another in which lymphadenopathy is most prominent. Leukemia with an acute course was first reported by Friedreich<sup>8</sup> (1857). Ebstein<sup>6</sup> (1899) described the outstanding symptoms of acute leukemia. It was not until Ehrlich's blood-staining methods came into use that specific types of leukocytes were related to the different clinical syndromes. With Naegeli's recognition of the myeloblast in 1900<sup>20</sup> and with Reschad and Schilling-Torgau's description, in 1913,<sup>23</sup> of what these authors thought was monocytic leukemia, the major divisions of the leukemias, as we now categorize them, were reasonably complete.

The term "multiple myeloma" was suggested in 1873 as the result of Rustizky's pathologic studies of a disease characterized by softening and fractures of bones and the presence of a peculiar protein in the urine.<sup>25</sup> The latter had been described as early as 1845 by McIntyre, Dalrymple, Watson, and Bence Jones.<sup>9</sup> Multiple myeloma was further characterized by the studies of Kahler (1889) and Bozzolo (1897). Wright (1900) drew attention to the similarity between plasma cells in bone marrow and the cells making up the tumors of multiple myeloma.<sup>9,25</sup> The association of multiple myeloma with hyperproteinemia was first noted by Perlzweig (1928) and elaborated by Magnus-Levy (1938).<sup>18</sup>

The presence of a cold-precipitable plasma protein was described by Wintrobe and Buell (1933)<sup>30</sup> Introduction of serum protein electrophoresis by Tiselius led, in 1939, to Longsworth and coworkers' description of the serum protein "spike" typical of multiple myeloma.<sup>17</sup> Since then, continuing refinement in methods of examination of proteins in serum and urine has permitted the recognition of a number of diseases characterized by overproduction of immunoglobulins or fractions thereof (Chapters 52 and 53).

Thus, by the first part of the 20th century the framework for the major subdivisions of neoplastic diseases of hematopoietic tissue had been developed. Discussions concerning further subdivisions of major categories continue to the present time and probably will persist until the etiology and pathogenesis of these diseases are better understood.

Leukemia is unquestionably divisible into granulocytic and lymphocytic varieties. These in turn may be separated into acute and chronic forms. Since, as the result of improved therapy (Chapter 47), some patients with acute lymphoblastic leukemia may live longer than patients with chronic myelocytic leukemia, the designations "acute" and "chronic" are no longer applicable to life span.<sup>2</sup> Nevertheless, they are still retained since they are appropriate with respect to the rapidity of development of symptoms, signs, and complications in these forms of leukemia. The use of the term "subacute" is discussed in Chapter 47.

The lymphomas are conveniently separated into two broad categories, HD and NHL. Hodgkin's disease has been further subdivided according to histologic patterns because these are of prognostic significance (Chapter 50). Subdivisions of NHL are discussed in Chapter 51. Mycosis fungoides is difficult to define as a distinct pathologic entity (Chapter 51).<sup>4</sup> The distinctive geographic distribution and exquisite sensitivity to therapy of Burkitt's (African) lymphoma justify its consideration as a separate entity (Chapter 51). Whether histiocytic medullary reticulosis and the ill-defined group of diseases often regarded as reticuloendothelioses

should be considered as lymphomas is debatable. However, since many authorities consider them to be neoplastic diseases of hematopoietic tissue, they are discussed in Chapter 51.

The immunoglobulin-secreting tumors differ somewhat from one another in their clinical and laboratory manifestations as well as with respect to the type of protein produced by the immunocyte clone (Chapters 52 and 53).

Other diseases discussed in this volume that are considered neoplastic in nature by some investigators are polycythemia vera (Chapter 30) and idiopathic myelofibrosis with myeloid metaplasia (IMF), as well as certain related diseases (Chapter 57).

## Interrelations of the Diseases

A number of aspects of the neoplastic disorders of the hematopoietic system suggest interrelationships or even "conversion" from one disorder to another.

"Conversion" from one to another disease most commonly is manifested by a change in the pathologic appearance of lymph nodes or by changes in the blood (leukemic conversion). In the latter situation, the blood is invaded by lymphoma cells or a leukemia-like picture develops in patients with polycythemia vera or myelofibrosis.

As lymphoma progresses, the architecture may change. The frequency with which a follicular pattern leading to classification as nodular lymphoma changes to a diffuse pattern has led many pathologists to disregard this as a distinct entity. In another form of conversion, lymph nodes from patients with HD in which the initial pattern is one of lymphocyte predominance may become progressively depleted of lymphocytes if cure has not been achieved (Chapter 50).

A leukemic pattern morphologically and clinically similar to that of CLL or ALL may develop in any type of non-Hodgkin's lymphoma (Chapter 51). Leukemic conversion is more common in children than in adults and

in children the leukemia is more likely to be lymphoblastic than lymphocytic.<sup>45</sup>

Blastic transformation of CML is the cause of death in most patients with CML (Chapter 48). Patients with CML may also develop marrow fibrosis similar to that seen in IMF (Chapter 48). Patients with polycythemia vera (PV) (Chapter 30) and, more often, those with IMF (Chapter 57) may develop a picture resembling CML or they may die with a blastic transformation. Rarely, polycythemia may develop in patients presenting with a CML- or IMF-like disease.<sup>42</sup> Sequential conversion from CML to PV, to IMF, to terminal AML has been noted.<sup>43</sup> The possible development of histiocytic neoplasms in CML<sup>31,41</sup> is discussed in Chapter 48.

The individuality of stem cells for the lymphocytic and myeloid cell lines (Chapter 2) is borne out by the rarity with which a myeloid neoplasm is converted to a lymphoid one, or vice versa. Development of AML or its variants has been observed in at least 14 patients with HD<sup>33</sup> and in 31 with myeloma or related diseases.<sup>44</sup> However, such patients usually had been treated by irradiation in large doses or with radiomimetic drugs so that such conversion may represent induction of leukemia by known leukemogens (page 1455) rather than the natural history of the disease. An excess of the occurrence of second malignant conditions has been noted in HD patients treated by either chemotherapy or radiation therapy and particularly in those treated by both modalities.<sup>32</sup>

The significance of reports such as that of ALL terminating in a picture resembling histiocytic medullary reticulosis<sup>35,38</sup> remains to be clarified.

The existence of changes such as those described above and the observation that some cases are difficult to categorize led Dameshek to suggest that these conditions be termed "myeloproliferative" or "lymphoproliferative" disorders<sup>38,39</sup> according to whether or not they seemed to originate from myeloid or lymphoid tissue, respectively. This is an interesting, broad descriptive concept, but, unfortunately, these terms have been misused by some physicians to imply a diagnosis.

## Incidence

Death rates from all types of cancer in the United States' population, 1950 through 1967, have been compiled.<sup>34</sup> Leukemia, lymphoma, and myeloma are responsible for approximately 10% of all cancer deaths (Table 46-2). The incidence of leukemia and lymphoma reported from Denmark, 1943 to 1957, is remarkably similar<sup>36</sup> to that in the United States. Incidence calculated by deaths, however, fails to include any cured patients. Consequently, the incidence of HD must be somewhat in excess of the figures given in Table 46-2.

At the present time, acute and chronic leukemia appear to be equally frequent and CLL is slightly more common than CML (Table 46-2) (Fig. 46-1). Leukemia and lymphoma are approximately equally frequent. Reporting of cell types varies so much from institution to institution that, except for noting that less than half of all lymphomas are HD, little can be said for their exact frequency.

### Changing Incidence of Disease

Steadily increasing mortality rates for leukemia in general were reported for the United States and other countries for many years.<sup>40</sup> This is evident between 1950 and 1958, as shown in Table 46-2. The rise has apparently slowed or ceased in the white population of the United States. Evidence for stable rates of mortality for leukemia among children in Connecticut between 1945 and 1959 and for all types of leukemia in Cornwall from 1948 through 1959 confirms the stability of present leukemia rates.<sup>40</sup> Whether the reported rising mortality of years past was real or artifactual is difficult to determine. The influence on mortality statistics of improved medical care, increasing use of laboratory diagnosis, improved disease certification, some increase in life span, and the fairly high frequency of leukemia in the elderly must be considered in evaluating the data. Features suggesting that leukemia may have been a less common disease in years past are the magnitude of the

Table 46-2. Death Rates for Leukemia and Lymphoma in United States Population, 1950, 1958, and 1967  
(Deaths per 100,000 Population, Age Adjusted)\*

	Non white Male			Non white Female			White Male			White Female		
	1950	1958	1967	1950	1958	1967	1950	1958	1967	1950	1958	1967
All cancer	144.5	170.9	218.0	144.1	140.2	143.4	157.9	166.6	181.2	139.3	129.4	125.7
All leukemia†	4.1	5.8	6.8	3.0	3.5	4.2	7.4	8.8	9.0	5.3	5.9	5.7
Acute		1.8	2.5		1.6	1.7		3.4	3.8		2.4	2.7
Chronic myelocytic		1.1	1.1		0.8	0.8		1.4	1.4		1.0	0.9
Chronic lymphocytic		1.7	1.9		0.8	0.9		2.4	2.5		1.2	1.1
All lymphomas‡	4.9	7.4	9.2	3.1	4.4	5.8	8.6	8.4	10.0	4.2	5.8	6.7
Hodgkin §	1.4	1.8	1.7	1.0	0.8	0.8	2.2	2.2	2.2	1.2	1.4	1.4
Lymphosarcoma	1.0	1.4	1.8	0.8	0.7	1.0	2.1	2.4	2.5	1.4	1.8	1.7
Reticulum cell sarcoma	0.3	0.6	0.9	0.2	0.4	0.6	0.5	0.8	1.4	0.3	0.6	1.0
Myeloma	1.1	2.7	3.4	0.8	1.9	2.4	1.0	1.7	2.2	0.7	1.1	1.5

\*Based on data from Burbank 31

†Derived by Burbank<sup>31</sup> as reported as "white, Mexican, Puerto Rican, or Cuban"; non white as "all others "

‡1950 rates for leukemia type not shown due to reporting change

§ Includes multiple myeloma



## AGE AND SEX OF 565 PATIENTS WITH LEUKEMIA

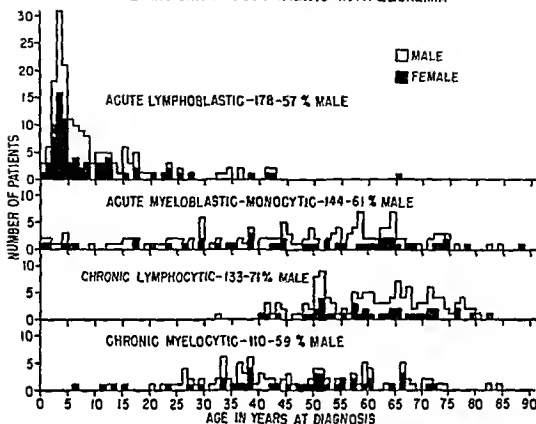


Fig 46-1. Types and age and sex distribution in 565 patients with leukemia examined in the authors' clinic (From Boggs et al,<sup>33a</sup> courtesy of the authors and Williams & Wilkins Company)

rise, a seemingly disproportionate increase in certain types of leukemia, and some evidence for changing sex differences.<sup>40</sup> The validity of the statistics is further supported by the observation that the incidence of childhood leukemia increased significantly in Israel between 1950 and 1960, while the lymphoma rate was constant.<sup>46</sup>

There is a suggestion of rising rates of NHL, but rates for HD appear quite stable (Table 46-2). The reported death rate from multiple myeloma has more than doubled between 1950 and 1967, a change that must reflect, at least in part, the widespread current use of protein electrophoresis as a diagnostic test.

#### Age Incidence

Age specific death rates for all of these diseases except acute lymphoblastic leukemia

increase dramatically and fairly steadily after the fifth decade of life has been reached.<sup>34</sup> The age specific rate for all forms of leukemia is shown in Figure 46-2. In the white male population, the rate decreases from 4.6 to 2.1/100,000 between the first and third decades. This early peak reflects the peak of ALL seen at age three to four years (Fig. 46-1). ALL becomes quite uncommon by the fourth decade (Chapter 47). Conversely, CLL is virtually unknown in persons younger than 30 years. AML and CML can occur in persons of any age, but become progressively more frequent as the population reaches middle age.

Hodgkin's disease has a somewhat peculiar age distribution in that there is a steady rise in incidence during the first three decades of life, the rise then slowing for two decades and again increasing to a peak in the seventh decade (Fig. 46-3). The early peak is more

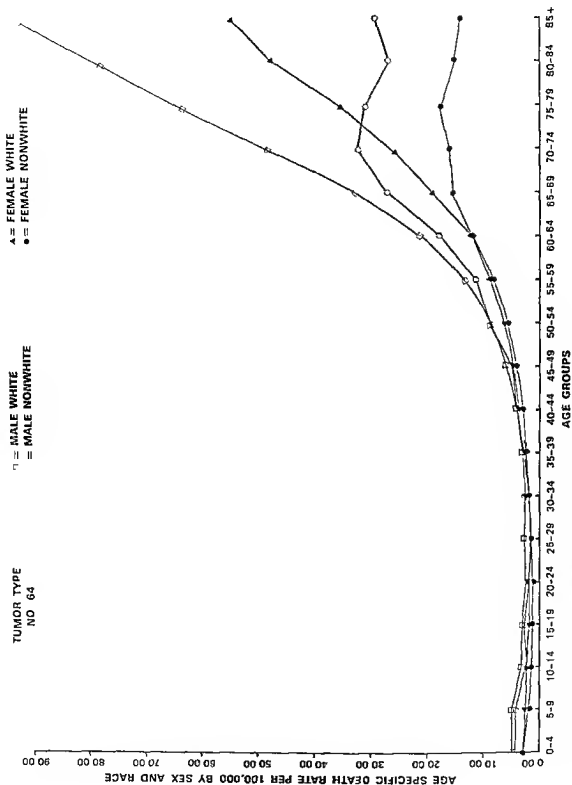


Fig 46.2. Age specific death rate from leukemia in the United States population. (From Burbark,<sup>34</sup> courtesy of the author and National Cancer Institute)

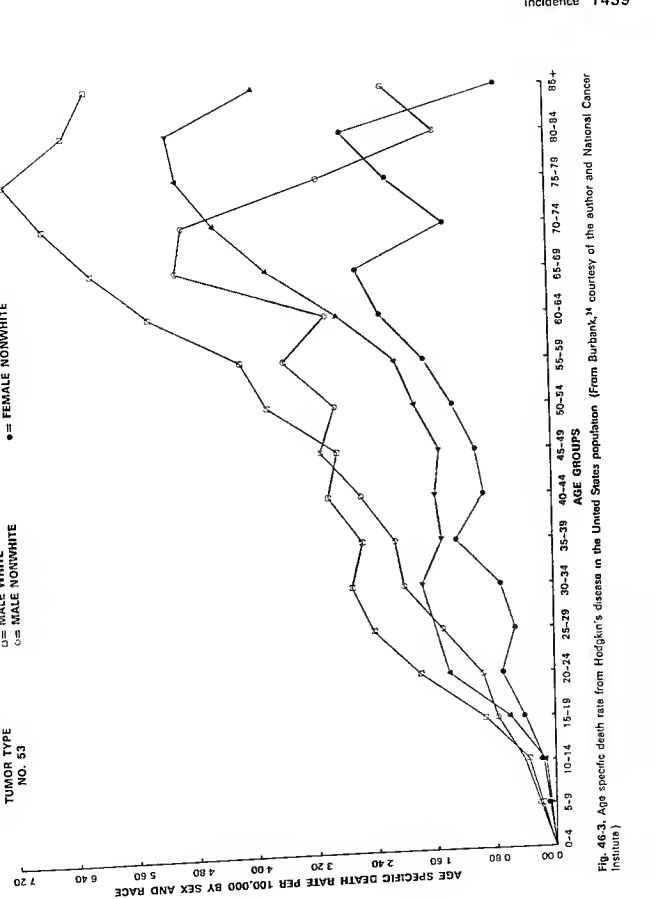


Fig. 46-3. Age specific death rate from Hodgkin's disease in the United States population (From Burbank,<sup>14</sup> courtesy of the author and National Cancer Institute)

pronounced in women than in men. This distribution has led to the hypothesis that the two peaks represent different types of disease<sup>37</sup>; the nodular sclerosis type (Chapter 50) may account for the early peak.<sup>45</sup>

NHL is observed in persons of any age, although the follicular forms are rarely seen in children.<sup>45</sup> The incidence is virtually the same throughout childhood and adolescence, but rises progressively thereafter.<sup>34</sup> Multiple myeloma rarely is found in persons younger than 30 years and reaches a peak incidence in those in their seventh decade, after which the rate declines.<sup>34</sup>

Since the size of the population at risk changes with age, age specific rates are not reflected in age frequency at diagnosis. Thus, most patients with CML or AML are found to be in their middle years (Fig. 46-1) if calculations are made according to age specific rate and population size.

### Sex Ratios

All of the neoplastic diseases of the hematopoietic system are more common in males than in females (Table 46-2) (Figs. 46-1, 2, and 3). Male-to-female ratios range from approximately 3:2 for acute leukemia, to 2:1 for CLL. The least degree of male-to-female preponderance is among children with ALL in whom the ratio is approximately 5:4.<sup>34</sup> In most of the diseases under consideration the rate of increase in later decades of life is faster for males than for females, accentuating sex differences as age increases.<sup>34</sup>

### Pathogenesis

All of the neoplastic diseases of the hematopoietic system represent disordered cell growth. In this section, data relating to the following questions are reviewed: Are these tumors unicentric (clonal) or multicentric in origin? Are the cells abnormal by functional or biochemical definition? What are the cellular growth rates and life spans (kinetic data)? Are control mechanisms for cellular growth intact or aberrant?

The primary cell involved in the malignant process in LSA, CLL, and ALL is presumed

to be the morphologically identifiable lymphocyte or lymphoblast. Since the plasma cell may not be a dividing cell (Chapter 7) a lymphoid precursor may be responsible in MM. Of the mixed cellular accumulation observed in HD, the Reed-Sternberg cell and its presumed relative, the histiocyte, are considered to be the "malignant" cells. In CML and AML there is evidence to suggest that the defect lies not in the myeloblast but in a pluripotent precursor (see Chapter 2 for normal stem cell systems). The Ph<sup>1</sup> chromosome defect described in the majority of patients with CML (page 1441) is found in neutrophil, eosinophil, erythrocyte, and platelet precursors. It seems reasonable to suggest that CML represents a stem cell defect in which increased and probably uncontrolled cell flow into neutrophil, eosinophil, monocyte, basophil, and platelet compartments accounts for the increase of these cells in the blood, while decreased flow into the red cell compartment results in anemia. In AML there is a flow into the myeloblast compartment, but it is abortive in that maturation is incomplete and there is decreased flow into the platelet and red cell compartments.

### Evidence for Clonal Disease

The pattern of spread of HD suggests that it may be clonal in nature and strong evidence for clonal origin can be marshalled for MM and CML and, to some degree, for the other neoplastic diseases of the hematopoietic system.

The homogeneous protein produced in MM contains either kappa or lambda light chains (Chapter 52) rather than the mixture of the two types found in normal immunoglobulins (Chapter 7). Although alternative hypotheses could explain this observation it is most simply interpreted on the assumption that MM begins in a single cell that produces a single type of light chain, the disease resulting from multiplication of that single cell. When two homogeneous proteins have been present, both have had homogeneous light chains, suggesting the presence of two distinct clones<sup>136</sup> (Chapter 52).

Evidence that CML is clonal is quite

strong and is derived from chromosomal (below) and cellular isozyme studies. Patients heterozygous for the A and B isozymes of 6-phosphogluconate dehydrogenase are found to have approximately equal representation of the A and B isozymes in their normal tissues.<sup>95,96</sup> When such heterozygotes have developed CML, analysis of their myeloid tissue reveals only one isozyme, A or B, while their nonleukemic tissues still contain an approximately equal mixture of A and B.<sup>95,96</sup> Interpreted most simply, these data suggest that CML begins in a single cell in which genetic expression of one isozyme is repressed or deleted.<sup>96</sup>

The clonal origin of AML from a pluripotent stem cell finds support in chromosome studies (page 1443). Additional evidence is found in studies suggesting that there are two separate and distinct red cell populations in patients with DiGuglielmo's syndrome, which differ with respect to content of A<sub>2</sub> hemoglobin, surface antigens, and enzyme content.<sup>130</sup> Again, certain patients with AML have a subpopulation of red cells abnormally susceptible to in vitro lysis.<sup>81</sup> In another study, abnormal DNA synthesis was described.<sup>81</sup> These observations suggest, but do not prove, that a pluripotent stem cell clone is producing abnormal red cells, although normal stem cells persist and produce normal red cells as well.

That CLL may be clonal in origin is suggested by the observation that lymphocyte surface immunoglobulins, often IgM protein,<sup>52</sup> may be homogeneous for kappa or lambda light chains.<sup>64,178</sup> Surface or even unreleased, intracellular homogeneous proteins are often present, even when such proteins are not detectable in serum.<sup>124</sup>

Examination of 33 of 34 patients with Burkitt's lymphoma suggested a clonal origin of the tumor by isozyme criteria and evidence for clonal origin was obtained in 92 out of 95 cases by immunoglobulin markers.<sup>96a</sup>

### Chromosome Abnormalities in Tumor Cells

A wide variety of chromosome abnormalities (Chapter 2) have been described in the

leukemias and lymphomas.<sup>186</sup> These chromosome abnormalities apparently are limited to the tumor cells since normal somatic cells such as skin and buccal mucosa are diploid and manifest no morphologically identifiable defects in individual chromosomes.

### Chronic Myelocytic Leukemia

In 1960, Nowell and Hungerford<sup>157</sup> described an abnormally small chromosome in patients with CML, the *Philadelphia (Ph)* chromosome. Subsequent studies demonstrated that 80 to 90% of patients with a "typical" clinical picture of CML have this defect (Chapter 48).

The Ph<sup>1</sup> chromosome<sup>186</sup> is a G-group chromosome, number 22,<sup>163</sup> which has lost a portion of its long arms. It is generally believed that the lost portions have been translocated rather than having been lost and there is evidence to suggest that the translocation is to chromosome number 9.<sup>179</sup> The amount of missing DNA, while averaging 30 to 50%,<sup>181</sup> seems to vary from patient to patient as judged by morphologic studies. Whether such variation is real or artifactual must await more accurate methods of measuring DNA per chromosome. If such variation does exist, it is conceivable that patients with typical CML, but without a morphologically identifiable Ph<sup>1</sup> chromosome, may in fact lack a very small, morphologically undetectable, portion of the long arms. The Ph<sup>1</sup> chromosome is present in marrow cells, but not in lymphocytes, so direct metaphase smears of marrow or blood rather than blood cultured in phytohemagglutinin should be studied in order to detect its presence.

The Ph<sup>1</sup> chromosome is found not only in neutrophilic granulocytic cells, but also in eosinophils,<sup>132</sup> normoblasts, and probably in megakaryocytes.<sup>212</sup> As noted in Chapter 2, this observation suggests that these cell lines share a common stem cell. The abnormality, when present, is found in virtually 100% of marrow cells in most patients. In occasional ones the Y chromosome also is lost so that the cells are hypodiploid.<sup>167</sup> Other changes may occasionally be present in the absence of blastic transformation.<sup>166,186</sup> That the de-

fect is acquired during the evolution of CML rather than being inherited is borne out by its absence in unaffected members of identical twins.<sup>87,110,111</sup>

These observations suggest that CML is a clonal disease; that is, it begins with a single, abnormal stem cell that has characteristics allowing it to proliferate in preference to normal stem cells. However, residual normal hematopoietic stem cells that do not contain the  $\text{Ph}^1$  defect are demonstrable in some patients with CML.<sup>83</sup> The abnormality does not disappear with therapy in most instances. Reports of its disappearance from the marrow of patients treated until severe pancytopenia was induced<sup>117,200,209</sup> do not allow determination of whether metaphases from myeloid cells or residual lymphocytes were studied. However, one patient has remained in remission for more than a decade with only a persistent, minor  $\text{Ph}^1$  marrow population.<sup>88</sup> Additional evidence for the clonal nature of CML, as judged by chromosome changes, is found in studies of a 69 year old phenotypically normal male who was a sex chromosome mosaic.<sup>101</sup> Barr bodies (Chapter 2) could be found in his neutrophils. Lymphocytes and marrow cells were chromosome mosaics of XY and XXY. However, the  $\text{Ph}^1$  chromosome was seen only in marrow cells, specifically the XY cells, and did not involve the XXY line in the marrow.

Radiation leads to an increased frequency of CML as well as AML (page 1456). Patients developing CML after irradiation exposure have been found to have the  $\text{Ph}^1$  defect.<sup>20</sup> Perhaps of even greater interpretive value with respect to the significance of this defect is its presence in patients exposed to irradiation who had not, at the time of the reports, developed CML.<sup>109,126</sup> If these patients should develop CML, it would strengthen the case that this defect is basic in the pathogenesis of the disease.

In contrast to the usual finding that the  $\text{Ph}^1$  abnormality is limited to myeloid cells, a family has been observed in which this defect was inherited and thus was present in all cells.<sup>217</sup> The presence of CML in two of these family members and unexplained neutrophilia in a third provide support for the

thesis that this defect may cause CML. However, in other families in whom more than one member developed CML, evidence for  $\text{Ph}^1$  abnormality was not found.<sup>58</sup> It must also be considered possible that the  $\text{Ph}^1$  abnormality, rather than being the cause of CML, is produced by the agent or mechanism causing CML.<sup>186</sup> For example, an oncogenic virus might produce a reproducible chromosome defect in any cell that it infects.

The  $\text{Ph}^1$  change has been observed in a few patients with seemingly typical AML, erythroleukemia, myelofibrosis, "essential thrombocythemia," and polycythemia vera.<sup>186</sup> Since CML can evolve into a picture virtually indistinguishable from at least the first four of these diseases (page 1435) it can be argued<sup>214</sup> that the cases represented undetected CML. Nevertheless, in the absence of firm knowledge of the role of the  $\text{Ph}^1$  chromosome in CML, the significance of its presence in patients with these diseases remains uncertain. For one thing, it may not be the identical defect in all these conditions, despite morphologic similarity. Conversely, the same defect might lead to different aberrant cellular expressions in different individuals, a concept compatible with suggestions that all of these diseases are very closely related (page 1434).

#### *Chromosome Changes during the Blast Phase of CML*

A variety of aneuploid and other random defects fairly typical of the defects observed in AML (see below) have been observed during blastic transformation of CML (Chapter 48). However, the most intriguing observation during blast crisis is the frequency with which a second  $\text{Ph}^1$  chromosome has appeared.<sup>80,186</sup> The number of second  $\text{Ph}^1$  chromosomes has decreased with remission and again has risen with return of crisis.<sup>80,205</sup> This suggests, but does not prove, that the blast crisis is the result of a new clone of cells arising within the leukemic population. Since the changes during the blast crisis of CML usually follow prolonged therapy, one may ask whether the changes represent the natural evolution of this disease or are the result of therapy. The infrequency of new abnormali-

ties developing in AML after prolonged therapy<sup>186</sup> suggests natural evolution.

### Acute Leukemias

As in CML, karyotypic changes, when present, appear to be limited to marrow and/or lymphoid cells involved in the acute leukemic process. Karyotypic changes are demonstrable in approximately 50% of patients with acute leukemia (either AML or ALL) (Table 46-3).<sup>101a,128,186,213,214</sup> This number suggests that if these are clonal diseases, the abnormal clones may not be expressed in a morphologically identifiable karyotypic form in all patients. Aneuploidy is the most common finding in acute leukemia and is unusual in other disturbances of the hematopoietic system except in congenital diseases. Its presence in the majority of cells from direct preparations of marrow or blood is strongly suggestive of acute leukemia; a few aneuploid cells, however, in the presence of a predominant diploid line are commonly found in marrow from patients without hematologic disease.<sup>162</sup> Certain refractory anemias have been reported to be associated with aneuploid changes similar to those found in AML.<sup>119,186</sup> Some such patients, especially those with sideroblastic anemia,<sup>132,184</sup> have proved to be "preleukemic," but many have not.<sup>143,184</sup>

The findings in ALL differ somewhat from those in AML (Table 46-3). Aneuploidy is almost always of a hyperdiploid nature in ALL,<sup>101a,213</sup> while hypo- and hyperdiploid cell lines are found with approximately equal frequency in AML. The autosomal group involved in duplication or omission is not the same from one patient to another. However, C and G group chromosomes are involved more often than are F group chromosomes.<sup>101a</sup> One patient with AML was found to have a tetraploid line of cells (92 chromosomes).<sup>210</sup> In addition, abnormal-appearing chromosomes may be observed.<sup>128,170,186</sup> In individual patients the same chromosome abnormality may reappear with relapse, after having disappeared during remission.<sup>186</sup> In others, different lines may appear with relapse.<sup>62,101a</sup> If no chromosomal changes

**Table 46-3. Chromosome Number in Acute Leukemia\***

Modal Number of Chromosomes (% of Patients)	Acute Myeloblastic (113 Patients)	Acute Lymphoblastic (106 Patients)
Hypodiploid	28%	1%
Diploid	50%	51%
No abnormality	(36%)	(41%)
Aneuploid subline	(14%)	(10%)
Hyperdiploid	22%	48%

\*Adapted from Sandberg and Hossfeld.<sup>186</sup>

are present initially in acute leukemia, they rarely develop, even with prolonged therapy.<sup>128,186</sup> Variants of AML, such as the DiGuglielmo syndrome, manifest chromosomal changes indistinguishable from those of AML.<sup>122,129,186</sup>

Unlike CML, in which the Ph<sup>1</sup> chromosome is present in virtually all marrow cells, some cells with normal karyotype are often present in acute leukemia, especially in ALL.<sup>101a</sup> This, plus the disappearance of chromosomal abnormalities during remission, suggests that abnormal and normal cells coexist in acute leukemia. The significance of this, like the significance of the Ph<sup>1</sup> chromosome in CML, depends on whether the defect is responsible for or merely a manifestation of the disease.

In patients with what has been described as "chronic monocytic leukemia" (Chapter 48) there is a high frequency of deletion of the Y chromosome in marrow, but not in other cells. This suggests that the defect is acquired.<sup>123,153</sup>

### Protein-Secreting Tumors

Cultures of marrow as well as PHA-stimulated cultures of blood lymphocytes reveal chromosome abnormalities in the majority of patients with protein-secreting tumors.<sup>186</sup> Cells with abnormalities always coexist with karyotypically normal cells and normal cells usually are in the majority. Since there has been no way to determine the origin of the

karyotypically abnormal cells the suspicion that they are tumor cells has not been confirmed. Although a variety of structural abnormalities as well as various types of aneuploidy have been described, approximately 80% of patients with macroglobulinemia and 30% of those with myeloma have a supernumerary group A size chromosome (Chapter 2). However, the morphologic characteristics of the extra chromosome have varied considerably from patient to patient. These abnormal chromosomes have not been found in fibroblasts<sup>93</sup> nor in a healthy monozygotic twin of an affected patient,<sup>201</sup> thus suggesting that this is an acquired defect.

### Chronic Lymphocytic Leukemia

An inherited abnormal G group chromosome, probably number 21, has been demonstrated in some families in which more than one member had CLL.<sup>100,186</sup> Since a similar abnormality has been found in individuals with no evidence of CLL the association may be fortuitous, rather than causally related.<sup>186</sup> Actually, data on chromosomes from patients with CLL are of questionable value. Their lymphocytes rarely divide in short-term culture, nor do they undergo blastic transformation when cultured with phytohemagglutinin (page 1448). Thus, reliable analyses of chromosome constitution of the lymphocytes involved in the CLL process are not available. The significance of occasional reports of random abnormalities,<sup>186</sup> such as abnormal frequency of aneuploid cells and chromatid breaks in patients in whom most metaphases were normal,<sup>219</sup> as well as the as yet unconfirmed report of a slight reduction in the size of small acrocentric chromosomes in males with this disease,<sup>99</sup> remains to be determined.

### Polycythemia Vera

Most patients with polycythemia vera are karyotypically normal. Of those in whom abnormalities have been reported, most had received extensive therapy, especially with <sup>32</sup>P and/or often their condition was evolving

into one simulating AML.<sup>186</sup> However, the finding of extra C group chromosomes in two untreated patients and a missing Y in another suggests that chromosome changes may sometimes occur in this disease, especially since the frequency of abnormal metaphases was found to decrease with therapy.<sup>133,186</sup>

### Lymphomas

Short-term cultures of lymph node cells from patients with *Hodgkin's disease* have shown a high frequency of hyperdiploid cells, but marrow cultures usually have given normal findings. Reed-Sternberg cells are polyploid under ordinary light microscopy, and whether or not the frequency of aneuploidy merely reflects the frequency of Reed-Sternberg cells remains a question.<sup>186</sup> A small proportion of cells with abnormal karyotypes have been reported in patients with *non-Hodgkin's lymphoma*. Most of these appeared to be randomly distributed and consisted of aneuploidy, or abnormally long arms were present on certain chromosomes.<sup>186,202</sup> Cytogenetically mutant clones have arisen in subcultures of an initially modally normal cell culture of Burkitt's lymphoma, but most direct cultures from patients with this disease have shown no cytogenetic abnormality.<sup>135</sup> Deletion of either the long or short arms of chromosome 17 or 18 in the E group has been reported in a few patients with NHL as well as in patients with Hodgkin's disease.<sup>151,194,202</sup> These chromosomes coexisted with cells of normal karyotype. Additional surveys will be required to determine if this abnormality is more frequent than other changes in these diseases. Hyperdiploid cells in direct marrow culture from patients with NHL have been observed to disappear with remission and to reappear with relapse.<sup>187</sup> Chromosome abnormalities were seen in tumor cells, but not in somatic cells in a patient from a family with a high incidence of lymphoma.<sup>131</sup>

In the disease or group of diseases that are considered under the heading of *myelofibrosis* (Chapter 57), chromosome changes rarely occur until the disease has evolved into a picture resembling that of AML.<sup>186</sup>



## Summary

Studies of chromosomes suggest, but do not prove, that CML, certain acute leukemias, the protein-secreting tumors, and perhaps certain lymphomas are clonal in nature. If this is so, there could be an initiating factor causing the production of the initial abnormal cell, or the chromosome abnormality may occur by chance. If an initiating factor, for instance, a virus, were incorporated into DNA, one might ask whether its persistence is required or is the genetic alteration perpetuated without viral persistence (page 1450). Certainly acquired chromosome abnormalities may disappear. In vitamin B<sub>12</sub> deficiency (Chapter 14), giant chromosomes and chromosomes with breaks are observed in metaphases from direct cultures of marrow and yet these disappear with B<sub>12</sub> therapy.<sup>131</sup>

An increased incidence of chromosome abnormalities, including some "clones," has been reported in healthy A-bomb survivors.<sup>68</sup> Chromosome aberrations are associated with virus-induced leukemia in mice. However, these are not detectable until the disease is fairly well advanced, suggesting that the chromosome changes are epiphenomena rather than essential to the development of leukemia.<sup>173</sup> In this regard it is of some interest that abnormalities similar to those induced by infection with SV 40 virus have been found in lymph node cells from patients with reticulum cell sarcoma.<sup>202</sup>

Abnormality of chromosome 21 or 22 in association with the leukocyte abnormalities of CML, Down's syndrome, familial CLL, and sideroblastic anemia when evolving into AML<sup>198</sup> would suggest that some essential control genes for leukocytes may be present in either or both of these chromosomes. On the other hand, the absence of leukocyte changes in two instances of monosomy of 21-22<sup>253,244</sup> casts doubts on this suggestion.

## Functional and Biochemical Abnormalities of Tumor Cells

Warburg's suggestion that all forms of cancer are characterized by an abnormally

high rate of aerobic glycolysis has not found scientific support.<sup>59</sup> However, Greenstein's hope<sup>59</sup> of finding an enzyme unique to cancer remains a viable hypothesis, at least in regard to RNA-dependent DNA polymerase (page 1454). Presently available data suggest that the cells involved in leukemia, lymphoma, and myeloma often are functionally deficient and biochemically abnormal.<sup>112,174</sup> It is not clear whether this reflects an intrinsic abnormality, a cellular response to an abnormal environment, or simply overgrowth of a normal subpopulation of cells whose characteristics differ from those of the major population. This interpretive dilemma is apparent in studies relating to CLL.

## Lymphocytes

The cell involved in CLL is the small lymphocyte. While subtle differences between its appearance and that of normal small lymphocytes may be noticed by light<sup>206</sup> or electron<sup>192</sup> microscopy, in most cases the cell appears normal. The normal function of the lymphocytic system is antibody production (Chapter 7) and evidence for defects in both humoral and cellular antibody systems is found in patients with CLL. Defective production of humoral antibody in response to antigenic challenge is present to a variable degree in most patients and is often reflected in hypogammaglobulinemia.<sup>153,197</sup> Skin reactions of delayed hypersensitivity are not lost,<sup>197</sup> but introduction of a new antigen may fail to elicit a positive skin response.<sup>67</sup> Blastic conversion in response to phytohemagglutinin (PHA)<sup>50</sup> or to allogeneic lymphocytes<sup>159</sup> is reduced in magnitude and is delayed as compared to that of normal lymphocytes, perhaps reflecting a decrease in surface receptor sites.<sup>156</sup> The response to PHA returns toward normal as blood lymphocyte concentration is reduced by therapy.<sup>50</sup> The chemical characteristics of the cells are similar to those of normal lymphocytes, but they live longer than normal lymphocytes in tissue culture and are abnormally susceptible to killing by adrenal corticosteroids and guinea pig serum.<sup>191</sup> Repair of x-ray- or ultra-violet-induced DNA damage

is enhanced in lymphocytes from patients with CLL as compared to those from normal persons,<sup>117</sup> and their RNA pattern differs.<sup>189</sup> Thus, there is evidence that the population of lymphocytes in patients with CLL differs significantly from normal.

However, it is known that the morphologically similar population of normal small lymphocytes is made up of functionally diverse subpopulations (Chapter 7). In normal individuals, some 25 to 50% of blood lymphocytes have a high density of surface immunoglobulin while the remainder have little or no detectable surface immunoglobulin.<sup>218</sup> The former cells are considered to be "bone marrow" (B) lymphocytes and the latter "thymic" (T), by analogy with similar studies in mice (Chapter 7). The percentage of "B" lymphocytes is markedly increased in many patients with CLL,<sup>52a, 172, 173, 178, 218, 219a</sup> while in some patients the cells are not identifiable as either "T" or "B" cells.<sup>178</sup> The relative decrease in "T" cells in such patients may explain their unresponsiveness to phytohemagglutinin since this response is considered a function of "T" cells. Thus, it cannot be determined if the excessive lymphocytes in this disease represent an abnormal or an expanded but normal population.

Although little is known concerning cellular chemistry in non-Hodgkin's lymphoma, immunologic defects similar to those found in CLL are common.<sup>152</sup> Blood lymphocytes from patients with NHL may fail to undergo blastic transformation with PHA,<sup>63</sup> suggesting that the circulating lymphocytes are involved in the disease. Extracts of tumor tissue from various types of lymphomas revealed excessive IgA, IgG, or IgM in some, but reduced immunoglobulin levels were found in others, suggesting that some might have arisen from "B" cells and others from "T" cells.<sup>207</sup> Consistent with this is the observation that lymphocytes in the circulation of patients with NHL represent an abnormally large proportion of either "T" or "B" lymphocytes.<sup>172</sup>

Anergy and loss of lymphocyte transformation are frequent in Hodgkin's disease.<sup>51,78</sup> Lymphocytes from patients with HD produce an abnormally large amount of

lymphotoxin in the absence of stimulation and an abnormally small amount when stimulated.<sup>189</sup> This could be interpreted as indicating that the lymphocytes may be abnormal in this disease. Alternatively, a specific lymphocyte compartment may be suppressed by the disease, as suggested by the fact that many anergic patients are lymphopenic. It would appear that loss of cellular immunity is selective, since circulating immunoglobulins are not usually reduced in HD.<sup>74</sup> Many investigators have assumed that the Reed-Sternberg cells and other histiocytes of HD are the "malignant" cells. However, except for the peculiar morphologic appearance of Reed-Sternberg cells, there are no data that define their characteristics except that they do not synthesize DNA (Chapter 50).

Normal immunoglobulins and immunoglobulin production in response to antigenic stimulation are reduced in myeloma. However, since normal immunoglobulins may increase in patients otherwise improved by chemotherapy<sup>54</sup> it is possible that the paraprotein acts as a suppressant. The frequently bizarre morphologic appearance of the myeloma cell suggests that it may be an abnormal cell, as does the abnormal pattern of methylation of tRNA.<sup>101</sup>

### Acute Leukemias

Many studies of cellular abnormality have focused on the acute leukemias.<sup>69, 118, 165, 211</sup> However, except for the PHA-transformed lymphoblast, comparison with normal cells is difficult since populations of normal "blasts" are not readily available. Consequently, whether biochemical differences between "leukemic" and normal leukocytes reflect true abnormalities of leukemic cells, or simply cellular immaturity, is difficult to determine. An RNA-dependent DNA polymerase is found in leukemic lymphoblasts and has not been unequivocally demonstrated in normal cells (page 1454). Both humoral and cell-associated immune mechanisms appear intact in ALL,<sup>92</sup> suggesting that the lymphoblastic proliferation neither represses nor replaces the normal immunocytic system.

The blasts of acute myeloblastic leukemia

often are abnormal in morphologic appearance. This is especially true of variants such as those present in myelomonoblastic leukemia and erythroleukemia. When some cytoplasmic maturation occurs it may follow an abnormal pattern such as the formation of *Auer rods*, an abnormality seen also, though rarely, in CML. These pink-staining, round, rod, or string-like cytoplasmic inclusions apparently are aberrant forms of azurophilic granules.<sup>103</sup>

Gavosto and associates<sup>107</sup> demonstrated decreased rates of protein synthesis in leukemic as compared to normal blasts. Evidence for *impairment of protein synthesis* in human acute leukemic blast cells was summarized by Gallo<sup>106</sup>: (1) there is direct evidence that protein synthesis is impaired in some leukemic blast cells as compared to normal blasts; (2) inappropriately methylated RNA precursors accumulate in leukemic cells and also in normal cells treated with protein synthesis inhibitors; (3) there are qualitative and quantitative differences in certain tRNA's in leukemic compared to normal blast cells which could cause or could be the result of impaired protein synthesis; (4) circular DNA dimers, found in leukemic, but not normal cells, can be induced in normal cells by inhibition of protein synthesis; (5) in leukemic cells, DNA synthesis and generation time are similar to or less rapid than those of normal cells and defective protein synthesis may impair DNA synthesis; (6) chloramphenicol is a potent inhibitor of mitochondrial protein synthesis and acute leukemia may follow chloramphenicol-induced aplastic anemia; however, (7) in many instances, morphologic and biochemical characteristics are similar in leukemic and in normal blast cells.

### Other Abnormalities

Regulatory mechanisms of cell differentiation may be recognized by measuring tRNA.<sup>75</sup> In stable cell systems, species specific tRNA methylase causes a highly reproducible sequence of methylation of tRNA, and quantitative and qualitative alterations in tRNA methylation occur in differentiating as

opposed to stable cell systems. That changes in tRNA methylation rate and sequence may have some relationship to cancer is suggested by the observation that certain alkylating carcinogens alkylate tRNA even more prominently than DNA.<sup>75</sup> Alterations in tRNA methylase specificity and tRNA methylase capacity have been demonstrated in a variety of neoplastic tissues, as compared to their normal counterparts, including those of human leukemia, Burkitt's lymphoma, myeloma, adenocarcinoma, and breast carcinoma. A species of phenylalanine tRNA, thought to be related to embryonic tRNA, was found in spleens from patients with CML, but not in normal spleens.<sup>154</sup>

Cells from the marrow of patients with AML may grow in culture, but, in general, just as *in vivo*, they fail to mature *in vitro*.<sup>77,88,111a,177</sup> This suggests that the defect responsible for lack of cellular maturation is found in the leukemic cell rather than in the environment of the leukemic host. However, other observations suggest that the question of cellular versus environmental abnormality<sup>146</sup> is unsettled. Thus, murine leukemic myeloblasts have been observed to mature *in vitro*, while failing to do so *in vivo*, and some *in vitro* maturation has been reported for human leukemic cells.<sup>97</sup> The few neutrophils that do mature in AML appear to be normal with respect to migration into exudates<sup>71</sup> and phagocytosis, but may be deficient in alkaline phosphatase activity.<sup>84</sup> From a functional viewpoint, cells in CML also probably are normal. Mature CML cells migrate normally into exudates<sup>71,73</sup> and are found to be efficient phagocytes when studies that correct for the number of immature cells are performed.<sup>84</sup> In most patients the Ph<sup>1</sup> chromosome is present in virtually all cells (page 1441) and leukocyte alkaline phosphatase is reduced in mature neutrophils (Chapter 48). Evidence for a membrane difference between mature neutrophils from normal persons and from patients with CML has been presented.<sup>60</sup>

A number of animal tumors contain a tumor-specific antigen, not demonstrably shared by any normal host cell and probably related to oncogenic viruses.<sup>79,141</sup> A search for tumor-specific antigen in leukemia has

sometimes been entirely unproductive,<sup>183</sup> but evidence for a neoantigen in leukemic cells has been offered. Lymphocytes from non-leukemic monozygotic twins recognized the lymphoblasts of their leukemic twin as foreign<sup>142</sup> and lymphocytes from patients in remission underwent blastic transformation when exposed to autologous leukemic cells.<sup>114</sup> Naturally occurring cytotoxic antibodies, apparently specific for leukemic cells, have been found in serum from normal persons and from relatives of leukemic patients,<sup>65</sup> and seemingly specific antisera against various types of leukemic cells have been produced by injecting the cells into primates<sup>150</sup> or by injecting Burkitt's lymphoma cells into rabbits.<sup>115,145</sup> Antisera prepared against lymph nodes from patients with Hodgkin's disease and absorbed by normal splenic tissue reacted with nodes from other patients with HD, but not with normal nodes, suggesting the presence of a tumor-specific antigen.<sup>159,161</sup> A few patients with lymphoma have carcino-embryonic antigen in their serum, an antigen common to several varieties of neoplasm.<sup>89,108</sup> By immunodiffusion techniques, but not by immunofluorescent techniques, CML cells were found to contain an antigen not demonstrable on CLL or normal leukocytes.<sup>220</sup>

### Cell Kinetics

In neoplastic diseases of the hematopoietic system there is an increase in the size of one or more *cellular compartments*; there may be an increase in an isolated subcompartment such as a lymphomatous lymph node or in a total body compartment as in leukemia patients and in most patients with myeloma. Compartment size represents the balance between cell inflow and/or cell division and cell loss by maturation or death. Thus, these tumors theoretically can be explained by increased cell reproduction, increased life span in the compartment, or combinations of the two.

The rate of cell reproduction is a function of three variables: (1) potential number of dividing cells in the compartment; (2) pro-

portion of those that are in a generative cycle; and (3) duration of the generative cycle. In the leukemias these parameters have been defined to some degree by means of tritiated thymidine-labeling studies.

The proportion of cells in a generative cycle usually has been found to be normal or slightly decreased in CML<sup>84,109</sup> and decreased in the acute leukemias<sup>140,191</sup> and in CLL.<sup>190</sup> In CML, the proportion of promyelocytes and myelocytes in cycle was found to be normal, while the cycling proportion of myeloblasts was decreased.<sup>84</sup> Estimated generation times have likewise been normal or longer than normal. From such studies, earlier concepts suggesting that leukemia is characterized by abnormally rapid cell proliferation have proved incorrect. However, uric acid excretion, which is a rough measure of the rate of overall cell turnover, is high in CML and the acute leukemias, but usually is normal in CLL.<sup>185</sup> From this as well as from estimations of the total number of dividing cells<sup>84,140</sup> it is apparent that overall cell production is increased in CML and in the acute leukemias. The increased cell production reflects an increase in the size of the potentially dividing compartment and is accomplished with a normal or subnormal proportion of the compartment in a generative cycle of normal or abnormally long duration. Whether total cell production is normal, subnormal, or slightly increased in the average patient with CLL cannot be determined from present data. However, careful kinetic analysis of data in two patients with "typical" disease revealed an overall increase in lymphocyte production.<sup>208a</sup>

The *life span* of leukemic cells also may be prolonged. In CLL there is evidence that the small lymphocyte has an abnormally long intermitotic interval, complementing data indicating that a reduced proportion of the compartment is in a generative cycle.<sup>190</sup> The life span of the lymphocytes, as measured by chromium labeling, is suggestively prolonged.<sup>221</sup> In CML, blood-labeling curves with  $^{51}\text{Cr}$ ,<sup>195</sup> or  $^{3}\text{H}$ <sup>84</sup> are quite prolonged. However, whether overall survival time from formation of the myeloblast

to loss of the mature PMN is prolonged is less certain.<sup>55,73,84</sup> The abnormal cell-flow patterns and distribution in CML (see below) complicate the interpretation of blood-leukocyte disappearance curves. A normal "life span" is difficult to assign to normal myeloblasts or lymphoblasts since they ordinarily divide again or mature rather than remain static in the compartment. Survival time for leukemic lymphoblasts and myeloblasts has not been well established. However, the studies of Mauer and coworkers<sup>140</sup> and of others suggest that leukemic blasts can move from a dividing compartment to a resting compartment only to again enter the dividing compartment some time later. This concept of "resting" leukemic blasts is important in designing chemotherapy schedules and is considered further in Chapter 55.

In addition to abnormalities of production rate and perhaps of cell life span, cells are *abnormally distributed* in the leukemias. In CLL, the proportion of lymphocytes that are normally recirculating (Chapter 7) is reduced and/or the time required for recirculation is prolonged<sup>76,121</sup>; output of cells from the thoracic duct is less than normal.<sup>66,190</sup> In CML, myeloid cells are produced in the spleen as well as the marrow and at times in the liver and other organs.<sup>84</sup> The undefined barrier (Chapter 2) to the escape of immature cells from the marrow is altered so that immature as well as increased numbers of mature cells are found in the blood. In a few patients observed by us in very early stages of CML, few or no immature cells appeared in the blood until the leukocyte concentration reached approximately  $50 \times 10^9/l$ , suggesting that loss of the barrier is a secondary rather than a primary pathogenetic mechanism. The normal flow of neutrophils is a one-way street, from marrow to blood to tissues or body cavities (Chapter 6). In CML, on the other hand, both mature and immature neutrophils may reverse the flow and reenter the marrow.<sup>84</sup> Furthermore, traffic between the blood and splenic tissue appears brisk. In the acute leukemias, cell production may occur in virtually any body organ (Chapters 47 and 54), but is most regularly found in

the spleen, liver, and lymph nodes in addition to marrow. In certain patients the barrier to immature cell release is intact and blasts are rare in the blood (aleukemic leukemia, Chapter 47), but in most patients it is not. Little is known concerning the traffic of blasts between blood and tissues,<sup>140</sup> but it is clear that blasts in AML may return to marrow from blood.<sup>135</sup>

*Dissociation between nuclear and cytoplasmic maturation* also may occur; "immature" CML cells may be phagocytic, in contrast to their normal counterparts.<sup>216</sup>

Thus, a variety of kinetic defects has been demonstrated in the leukemias. The cellular accumulation in blood and tissues reflects the summation of these defects.

The kinetics of the tumor cells in myeloma<sup>208</sup> and in the lymphomas<sup>158</sup> suggests that these are slowly growing tumors. However, the growth kinetics of normal cells, which are comparable to the tumor cells in these diseases, has not been measured. Consequently, one cannot be certain whether their growth rate is faster or slower than normal.

### Control Mechanisms

As noted in Chapters 2, 6, and 7, our understanding of how the compartment size of leukocytes is controlled is quite incomplete as compared to that of red cells (Chapter 4) or even platelets (Chapter 9). As a result, the long-standing hypotheses that leukemia, and for that matter lymphoma and myeloma, may be due to excessive levels of cell-growth stimulators, decreased levels of inhibitors, or other factors have not really been tested. There are some data, however, that bear on the question of maintenance of normal control.

As previously mentioned (page 1446), levels of normal immunoglobulin have improved following treatment of some patients with myeloma. This suggests that control mechanisms of immunoglobulin level (Chapter 7) are intact in myeloma and, if this is true, indicates that production of the myeloma protein is not subject to this normal control.

A factor that stimulates the growth of granulocytic colonies *in vitro* (colony-stimulating factor, CSF) can be demonstrated in normal human urine, plasma, and leukocytes.<sup>82,118</sup> Whether the substance is active *in vivo* or of physiologic significance remains to be determined. However, if CSF should prove to be a true neutropoietin, its progressive increase as the leukocyte count rises in CML,<sup>176</sup> its absence from some leukemic leukocytes,<sup>113</sup> and its erratic increase in acute leukemia<sup>102,148</sup> suggest abnormal and often physiologically inappropriate production of CSF in leukemia.<sup>146</sup>

Lymphocyte compartment size is controlled, at least in part, by the degree of antigenic stimulation (Chapter 7). Since patients with CLL, NHL, or HD often are unresponsive to such stimulation, some form of faulty control must be assumed.

## Etiology

The cause of leukemia or of lymphoma is unknown. However, certain factors that influence the frequency of development of these diseases in the population at large have been identified and a great deal has been learned concerning the cause of seemingly analogous diseases in animals.

Theories concerning the nature of leukemia, as well as cancer in general, can be divided into two broad categories: (1) the cell causing these diseases is intrinsically abnormal and all progeny of such cells must also be abnormal; (2) the cell is abnormal by virtue of its environment, and external influences lead to an abnormal pattern of growth.

In either case the disease could be clonal, i.e., the abnormality could begin in a single cell and the disease might be the product of that single cell (unicentric origin), or the abnormality could begin or be induced in more than one cell (multicentric). These and related topics have been discussed, under the heading "pathogenesis" (page 1440).

It is possible that these diseases do not have a demonstrable etiologic basis. Random genetic aberrations may occur in all dividing-cell systems, assuming that they are clonal.

When an aberration is viable and confers a selected advantage for growth as compared to normal cells, a tumor may result.

The causes of these diseases may differ; indeed, the observation that irradiation increases the frequency of certain leukemias but not others (page 1456) strongly supports this view.

## Viruses

The possibility that leukemia and lymphoma are due to infection was raised almost concurrently with their initial description. Indeed, CML can be mimicked by certain infections (Chapters 41 and 48); the granulomatous nature of HD certainly suggests a reaction to some organism. However, to date no organism has been clearly shown to have an etiologic association with hematopoietic neoplasia in man.

There is now no doubt that leukemia and lymphoma in mice, fowl, and probably various other species require the presence of a virus for their clinical expression.<sup>250,272,283</sup> In 1903, Borrel<sup>233</sup> suggested that cancer is due to a virus and, in 1909, Ellerman and Bang<sup>241</sup> demonstrated that fowl leukosis could be transmitted by a sub-(light) microscopic, filterable particle. Shortly thereafter, Rous described virus-induced sarcoma.<sup>282</sup> It was almost 50 years before the studies of Gross,<sup>219</sup> Friend, and others provided unequivocal data that a variety of animal leukemias are intimately and etiologically related to viruses.<sup>283</sup>

Leukemia in the laboratory mouse has become the classic model. In certain strains, leukemogenic virus is passed to offspring via infected ovum. Some data also implicate infected sperm.<sup>234</sup> The virus is introduced into newborn mice who suckle from mothers harboring the virus. Passage of virus to older normal mice, however, is quite difficult; consequently, evidence for "infectivity" in the usual sense with spread by respiratory, oral, or vector routes is tenuous. The data suggest that the virus is present in utero or early infancy rather than being acquired in later life. Yet, the incidence of leukemia in strains of mice harboring leukemogenic viruses differs. In some, the frequency of death from

old age or other causes far exceeds death from leukemia. Others, such as the AKR strain, usually will die of leukemia. Induction of viral-induced murine leukemia can be hastened by such factors as irradiation, immune system damage, or chemical leukemogens.<sup>251</sup> Thus, the mouse model suggests that the presence of virus is essential, but various genetic and environmental factors alter the rate of its expression.<sup>266</sup>

Viruses are distinguished according to whether the primary nucleic acid constituent is RNA or DNA. Most animal leukemia viruses are RNA viruses.<sup>283</sup> A particle ("*C*"-type particle) identifiable by electron microscopy has been shown to represent an RNA-type leukemia virus (oncornavirus) in one stage of development (page 1452). In the marrow of leukemic mice this type of particle often is abundant in megakaryocytes, but may be scant in leukocytes. At certain stages of disease it can be obtained from plasma by differential centrifugation.

### Explanations of RNA Virus Action

To explain RNA virus action, Temin<sup>287</sup> proposed that viral RNA can be converted to cellular DNA (*pro-virus*) by transcription and remain as part of that cell's genetic information, including the potential to produce more virus. Such conversion would presumably require an enzyme capable of translating RNA into DNA. He then demonstrated the presence of RNA-dependent DNA polymerase in Rous sarcoma tissue; Baltimore demonstrated it in Rauscher leukemia.<sup>290</sup> Temin showed that the Rous sarcoma virus can make a DNA copy of itself and that the DNA copy is a template for synthesis of new virus. Thus, genetic information, originating from RNA, is incorporated in the cell (*reverse transcription*).

At least 15 tumor viruses have now been shown to contain reverse transcriptase.<sup>283</sup> Thus, at least part of Temin's "provirus" hypothesis appears to be correct. In theory, once a cell has been infected and the viral information has been converted into DNA, reverse transcriptase is no longer necessary

because the provirus will be duplicated during DNA synthesis in the generative cycle and passed to daughter cells produced by mitosis. The "*oncogene*" theory of Huebner and Todaro,<sup>257</sup> on the other hand, suggests that the genetic component of most, if not all, vertebrates contains information for production of RNA-tumor viruses. Viral information ("*virogene*") and the portion of the viral information responsible for inducing malignant transformation of the cell ("*oncogene*") are inherited "vertically," but normally are repressed. Derepression of the "*oncogene*" may be the result of toxic insult (chemical carcinogens, irradiation, etc.), aging, or infection with other viruses or other factors and results in malignant cellular transformation. The "*oncogene*" could be derepressed, but the complete "*virogene*," necessary for viral reproduction, might not be derepressed and, consequently, viral particles might not be present. Temin's<sup>288,289</sup> "*proto-virus*" theory (not to be confused with his somewhat complementary "provirus" theory discussed above) is similar to the "*oncogene*" theory in that information for cancer (provirus) is transmitted vertically. It differs from the oncogene theory in postulating that proviruses play a role in normal cellular differentiation; that, at some stage in normal cellular differentiation, reverse transcriptase plays a role; and that, for the provirus to induce cancer, somatic mutation must lead to a modification of RNA, DNA, or reverse transcriptase. While certain data tend to support either the oncogene or provirus theory,<sup>283</sup> further studies are required to determine whether either is correct as presently formulated. It may be pointed out that these theories are compatible with either a clonal or a multicentric origin of hematologic tumors; that is, the event leading to the expression of viral tumorigenesis may begin in a single cell with subsequent proliferation of that cell, leading to a clonal tumor, or it may begin in many cells concurrently and lead to similar chromosomal and/or enzymatic changes in all affected cells so that they would be inferred, incorrectly, to be the progeny of a single, transformed cell.

Leukemia occurring spontaneously in mice and that induced by transplanting leukemic cells are both virus-related. Transplanted murine leukemia can be cured by a variety of cell-killing chemicals, while spontaneous leukemia is much more resistant. One explanation for this difference would be to suggest that, in transplanted leukemia, the virus, at least as reflected by viral replication, does not persist, while in spontaneous leukemia the virus persists and replicates, perhaps in cells other than those identified as leukemic, and reinfects the "leukemic" cells after therapy. Subtle antigenic differences between host and transplanted cells could represent alternative explanations for this difference between the responses of spontaneous and transplanted leukemia to therapy.

#### **Evidence for Viruses as Etiologic Factors in Leukemia and Lymphoma in Man<sup>224,233</sup>**

The unequivocal evidence of an etiologic relationship of virus to tumors in many species of lower animals strongly suggests the possibility of such a relationship in these diseases in man. However, despite intensive studies designed to demonstrate a viral agent in leukemia and lymphoma in man, conclusive evidence has not been forthcoming. The search for a human leukemia virus has centered around the following types of studies: attempts to demonstrate by electromicroscopy virus-like particles in human tissue or plasma, the possible emergence of virus in human cell lines in tissue culture; attempts to transmit disease by injecting cell-free human material into lower animals; and the identification of RNA-dependent DNA polymerase in human leukemic tissue.

#### ***Virus-like Particles in Patients with Leukemia and Lymphoma***

Particles resembling murine leukemia viruses, "C-type particles," have been reported in leukemic or lymphomatous cells examined by electron microscopy.<sup>239,295</sup> However, such particles have been quite sparse, and were

found in only a small proportion of the specimens examined. As the science of electron microscopy has evolved, the need for thin sections in order to avoid artifacts resembling such particles has been recognized and a number of investigators now consider earlier reports based on thick sections to be uninterpretable.<sup>280</sup> Using thin-section techniques, particles strongly resembling animal tumor virus, including "budding" in C-type particles, have been reported,<sup>239,295</sup> but careful surveys of tissue by other workers have failed to reveal such particles.<sup>278,280</sup>

When it was found that tailed, "C-type particles" could be spun from cell-free plasma of mice and other animals with virus-induced leukemia,<sup>227</sup> excitement was engendered by the identification of particles resembling C-type particles in spun plasma or urine pellets of patients with leukemia.<sup>229,262</sup> However, other studies<sup>235,274</sup> failed to disclose a higher incidence of such pellets in patients with leukemia as compared to that in controls. It is possible that the structures in question may be organelles of ruptured, normal blood cells.<sup>231,278</sup>

#### ***Virus in Cell Cultures***

Although other viruses have not been seen in many long-term cultures of lymphocytoid cells derived from patients with leukemia,<sup>252</sup> a herpes-like virus (the Epstein-Barr [EB] virus) has been demonstrated in cell cultures from many patients with Burkitt's lymphoma,<sup>235,242,253</sup> and in cell lines from patients with leukemia or with various cancers, as well as in cultures of presumably normal cells.<sup>273</sup> Even when herpes virus has not been seen in established lymphoid cultures, its presence could be inferred in most, if not all cultures, by means of antibody studies.<sup>284</sup> In fact, the most reliable means of establishing long-term cultures of normal lymphoid cells is to infect the cells with EB virus.<sup>245</sup> Thus, in a sense, the virus confers a "malignant" growth potential on normal cells. It stimulates DNA synthesis in infected cells, a property shared only by known oncogenic DNA viruses insofar as is known



presently.<sup>284</sup> Conceivably, this effect might be mediated by derepressing "proviral oncogenes" (page 1451) already present in the cell.

There is serologic evidence for the widespread distribution of the EB virus in the general population. In patients with Burkitt's lymphoma, including both African and American patients, antiviral titers almost always are present and the titers are quite high.<sup>254,264,284</sup> The EB virus appears to be the cause of infectious mononucleosis<sup>254,275,286</sup> (Chapter 43). While its etiologic role in Burkitt's lymphoma is not proved,<sup>276</sup> it may be asked whether the same virus can cause malignant and benign disease in different populations. In this regard it has been demonstrated that expression of infection with the murine, Friend leukemia virus is under the control of specific genetic loci.<sup>277</sup> Additional observations of possible significance are the fact that a cell line that produces tumors in hamsters has been derived from a cell culture originally obtained from a patient with infectious mononucleosis<sup>223</sup>; and that inoculation of a herpes virus induces a lymphoma-like illness in marmosets and lymphoblastic leukemia in owl monkeys.<sup>222,268</sup>

There is a higher than normal frequency of serologic evidence for EB virus infection in patients with lymphoblastic leukemia and lymphomas other than Burkitt's, but not in patients with CLL. However, neither the frequency nor the height of the titer approaches that seen in patients with Burkitt's lymphoma.<sup>263,269</sup> The development of infectious mononucleosis during the course of ALL<sup>269,285</sup> makes it most unlikely that EB virus causes ALL. It was suggested that a preceding infection with EB virus might somehow "trigger" the subsequent development of ALL.<sup>265</sup> However, a comparison of the frequency with which leukemia or lymphoma developed in veterans who did or did not have infectious mononucleosis during World War II revealed no differences between the groups.<sup>271</sup> An increased frequency of antibodies to herpes virus hominus type 2 has been reported in patients with HD.<sup>236</sup> The demonstration of an unidentified herpes-type virus in a culture of lymph nodes from

patients with HD<sup>240</sup> is still another intriguing, but as yet unproven, piece of evidence that these DNA viruses may play some role in neoplasia in man.

### Antiviral Antisera

The cellular specificity of antisera, prepared against known oncogenic viruses, provides indirect evidence for the viral origin of leukemia. Immunofluorescent antisera prepared against "pellets" from plasma of leukemic patients labeled leukemic, but not normal, leukocytes. The spectrum of activity of this plasma was quite similar to that of immunofluorescent antibody prepared against Rauscher murine leukemia virus.<sup>230</sup> Absorption of antipellet antibody with normal leukocytes did not remove the antibody's ability to label leukemic cells, but repeated absorption with normal bone marrow did,<sup>296</sup> suggesting that the antigenic difference reflected a difference between mature and immature cells rather than a tumor-specific antigen. Furthermore, anti-Rauscher leukemia virus antibody labels cells from patients with a wide variety of nonmalignant hematologic diseases.<sup>244,258</sup> Mice injected with Rauscher or Friend leukemia virus developed new cellular antigens in skin as well as leukemic cells.<sup>267</sup> These antigens were acquired whether or not the animal was susceptible to leukemia.

### Attempts to Induce Leukemia

Attempts to induce leukemia in sub-human primates by injecting adult, neonatal or in utero animals with cells or subcellular material from human leukemia or lymphoma or with viruses arising in tissue culture lines have been unsuccessful.<sup>260</sup>

### Infants Born of Leukemic Mothers

If leukemia is due to a virus, then infection of infants born of leukemic mothers would seem almost certain since most, if not all, human viruses cross the placenta, as does the murine leukemia virus.<sup>224</sup> Leukemia has de-

veloped in at least three children born of leukemic mothers,<sup>272,238</sup> but adequate follow-up of a series of such children has not been reported.<sup>229</sup> Passage of leukemic cells across the placenta has been suggested,<sup>279</sup> but quinacrine, the compound used as a cell label, elutes from leukocytes and, consequently, interpretation of the reported data is difficult.

### Transfusion of Cells from Patients with Leukemia

Leukemia has not been induced by transfusion of cells from patients with leukemia to nonleukemic subjects.<sup>247,291</sup> When large numbers of cells from patients with CML have been transfused into neutropenic patients with acute leukemia, transient engraftment of CML cells has been noted.<sup>186</sup> Considering our present knowledge of tissue transplantation in man and the nature of viral transmission in animals, these short-term observations are basically uninterpretable with respect to the etiologic background of leukemia.

*Transmission of human leukemia to normal human cells* has apparently occurred in two instances.<sup>273</sup> Children with leukemia were given total body irradiation, and marrow from a normal sibling of the opposite sex was transplanted. Leukemia recurred but the leukemic cells had the chromosome constitution of the donor, suggesting that the cell graft had acquired leukemia.

### Reverse Transcriptase in Human Tumors

Gallo and associates were the first to demonstrate reverse transcriptase (page 1451) in the blood cells of some patients with acute leukemia.<sup>283</sup> In their studies the enzyme found in the blood cells of these patients met all of the biochemical criteria for a true reverse transcriptase, whereas enzymes isolated from normal cells have not met these criteria.<sup>283</sup> For example, an enzyme from PHA-stimulated normal lymphocytes proved to be an RNA-primed, but DNA-directed

DNA polymerase rather than a reverse transcriptase. The reverse transcriptase may be derived from a tumor virus. An antibody against type C virus reverse transcriptase derived from sub-human primate sarcoma has been found to react with reverse transcriptase from human leukemia.<sup>283</sup> The human transcriptase produces DNA with some sequence homology to viral RNA of simian and murine sarcoma virus, as judged by hybridization studies.<sup>283</sup>

### Other Infecting Agents

The granuloma-like pathologic picture that is seen in HD led to an intensive search for organisms such as the tubercle bacillus in patients with this disease.<sup>256</sup> Although some positive reports appeared, to date there has been no convincing evidence of the association of any organism with such granulomas.

Some of the "viruses" isolated from leukemic tissue have proved to be mycoplasmas on further study.<sup>243,246</sup> In general, these have been thought to be secondary invaders of cell cultures rather than etiologically related to the disease.<sup>243,246</sup> Attempts to link leukemia and lymphoma in human beings to disease in pets have generally been unfruitful.<sup>405</sup>

### "Clustering" of Patients with Leukemia, Lymphoma, and Myeloma

If leukemia or lymphoma is induced by a communicable infectious agent or by some environmental factor that is not uniformly distributed, then a nonrandom distribution of cases in time and space would be expected. Apparent epidemics have been reported,<sup>343,363,402</sup> such as the development of acute leukemia in eight fairly closely associated children in Niles, Illinois, between 1957 and 1960,<sup>343</sup> and Hodgkin's disease occurring in 31 persons who could be interlinked by personal contact in one way or another, many of them through a New York high school.<sup>402</sup> However, the statistical significance of such reports is questionable. In this regard the

quotation from Plutarch cited by Ederer et al.<sup>324</sup> seems most applicable: "It is no great wonder if in long process of time, while fortune takes her course hither and thither, numerous coincidences should spontaneously occur."

In an attempt to determine if clustering does occur, the distribution of patients with leukemia in relation to known population density and the time of onset of disease in different cases with respect to one another, to season of the year, or to month of birth of the patients have been studied. The statistical methods of such studies, however, are complex and imperfect.<sup>323,333</sup> Some evidence for time-space clustering of leukemia has been obtained in Buffalo<sup>388</sup>; Northumberland and Durham<sup>364</sup>; Cornwall<sup>406</sup>; Liverpool<sup>376</sup>; urban, but not rural, Manitoba<sup>335</sup>; in children under six, but not older children<sup>333</sup> or adults,<sup>311</sup> in New Zealand; in children under six, but not in older children, in London<sup>399</sup>; in children, but not adults, in Georgia,<sup>325</sup> and in Portland, Oregon, but not in the state of Oregon as a whole.<sup>363</sup> Little or no evidence for time-space clustering of leukemia was obtained for San Francisco<sup>362</sup> and Connecticut,<sup>305,324</sup> but there was evidence for lymphoma clusters in Connecticut. No tendency for clustering of leukemia was detected in upper New York State,<sup>329</sup> Hawaii,<sup>367</sup> or Los Angeles.<sup>332</sup> Clustering in time and space has been reported for myeloma in Minnesota.<sup>365</sup>

Literature seeking to relate the month of the year to the onset of leukemia<sup>342,368</sup> or HD<sup>317</sup> is quite confusing. Certain studies have reported higher onset rates in certain seasons, but the peak season has differed from one study to another. Some studies suggest that the month of birth has some relation to the frequency of leukemia in the first few years of life,<sup>323,342,368</sup> but no such relation was detected in others.<sup>306,376,395,399</sup>

In summary, the literature dealing specifically with leukemia clustering is equivocal. Taken as a whole, it suggests that there may be a slight tendency for leukemia in children to cluster in time and space, but such a tendency seems limited to densely populated

urban areas. In a negative sense, the absence of strong clustering tendencies suggests that leukemia is not a communicable disease, at least as we commonly think of such diseases.

Examination of space distribution of United States mortality statistics<sup>34</sup> lends some support to a mild tendency for hematologic neoplasms to cluster. For instance, acute leukemia is significantly more common in New York and New England than in the Southeast. To some degree this probably reflects reporting accuracy since most of these diseases are more frequent in the Northeast and less common in the Southeast. However, it is difficult to apply this explanation to all differences since acute leukemia is also reported at less than average rates from the Midwest, while lymphoma and myeloma are reported at greater than average rates from the same region.

Clustering in space of Burkitt's lymphoma (Chapter 51) is most remarkable.<sup>235,311,312</sup> This tumor is found in a belt across Central Africa and, with the exception of New Guinea,<sup>28</sup> tumors of similar pathologic appearance, age incidence, and response to therapy have rarely been observed elsewhere. The increased frequency of Burkitt's lymphoma correlates very well with a median annual temperature above 60°F and median annual rainfall exceeding 20 inches. Tribal origin does not appear to be a factor in incidence. As noted on page 1452, there is some evidence for an association with a herpes virus and, since the climatic limits of the disease duplicate those of certain strains of mosquitos and flies, a vector-borne infection also has been suggested.

### Other Environmental Factors

As will be noted, a variety of environmental factors have been suggested as influencing the frequency of leukemia, lymphoma, and myeloma. However, with the exception of gamma irradiation and benzol and related hydrocarbons, no firm relationship of such factors to disease has been established.

Table 46-4. Irradiation Exposure and the Risk of Developing Leukemia

Type of Exposure	Leukemia Risk* (Frequency/Control Population)
Survivors of atomic bomb <sup>152</sup>	
Hiroshima	
> 100 rads exposure	23 6
5-99 rads exposure	3 7
Nagasaki	
> 100 rads exposure	10 3
5-99 rads exposure	< 1 0
Radiologists <sup>179</sup>	2 5
Patients receiving radiotherapy	
Ankylosing spondylitis	
> 2 000 rads	14 4
< 500 rads	4 2
Polycythemia vera	
<sup>32</sup> Phosphorus x-irradiation	13 7
	11 1
Chronic lymphocytic leukemia	
<sup>32</sup> Phosphorus	3 2
Thymic enlargement	71 0
Cancer of cervix <sup>150</sup>	71 0
Hyperthyroidism <sup>100</sup>	71 0
Diagnostic irradiation	
In utero	71 5
Postnatal	71 0

\*Calculated from data published by Lillienfeld<sup>179</sup> except where otherwise indicated. Insofar as possible, risk figures were derived from a control population with the same disease rather than from the general population.

## Irradiation

Exposure to gamma irradiation in appreciable doses leads to an increase in the frequency of CML and AML and perhaps ALL. There is some evidence to indicate that irradiation exposure may also induce small-cell LSA, HD, and myeloma, but, as yet, there is no evidence of any relation between irradiation and CLL or histiocytic lymphoma. Data upon which these conclusions are based, derived from surveys of survivors of atomic blasts, patients with various diseases treated

by irradiation, and radiologists exposed to irradiation, reflect the risk of developing leukemia following various forms and doses of irradiation. These are summarized in Table 46-4.

## Survivors of Atomic Blasts

Periodic surveys of the survivors of the atomic blasts in Hiroshima and Nagasaki have revealed a clear excess of leukemia, most notably CML and AML and probably ALL, but not CLL.<sup>306,309,318,343,352,374</sup> The incidence of leukemia has varied inversely with the survivor's distance from the blast hypocenter and directly with the calculated whole body dose (Table 46-4). When distance increased to the point that the calculated dose was reduced to less than 5 rads, the incidence of leukemia could not be distinguished statistically from that in unexposed populations. However, there is no clear evidence for a threshold exposure below which there is no increase in incidence.<sup>352</sup> The fact that the frequency of leukemia has been less in Nagasaki than in Hiroshima (Table 46-4) may reflect differences in type of irradiation, such as a lesser leukemogenic effect of neutron as compared to gamma irradiation, since the bomb directed at Nagasaki had a much higher neutron:gamma emission ratio than did that falling on Hiroshima.<sup>352</sup> It is of some interest with respect to the possible role of irradiation in leukemia in general that the course and clinical behavior of leukemia in A-bomb survivors have not been unusual; those with CML usually have had the Ph<sup>1</sup> chromosome and in those with acute leukemia the chromosome changes were fairly typical.<sup>360</sup> The peak incidence of disease probably occurred six to seven years after the blast, but an increased incidence as compared with that in a normal population has persisted for more than 20 years.<sup>374</sup> The rate of decline in incidence appears to be slower in those lightly than in those heavily exposed.<sup>369</sup> It has been suggested that this may be attributable to occupational exposure to benzol and to x ray.<sup>353</sup> No excess of leukemia has been found in children born of exposed survivors.<sup>346,354</sup>

Lymphosarcoma, HD, and myeloma also have been suggestively more frequent in heavily exposed survivors, but histiocytic lymphoma and CLL, the latter being a disease which is rare in Orientals (page 1461), have not.<sup>301</sup>

Chromosome changes are demonstrable in a minor population of lymphocytes in apparently healthy survivors, as they also are in patients exposed to total body irradiation.<sup>302</sup>

### *Patients Treated by Radiotherapy*

The terminal phase of polycythemia vera (Chapter 30) may resemble AML or CML, even if phlebotomy has been used for the treatment of the polycythemia. However, such a terminal leukemic picture is more frequent if x ray or <sup>32</sup>P therapy has been employed,<sup>179</sup> and there is a roughly linear relationship between the total dose of <sup>32</sup>P and the frequency of terminal leukemia. Chronic lymphocytic leukemia (Chapter 50) rarely, if ever, terminates in an acute phase unless large doses of irradiation have been employed in the treatment, and, in such patients, as in most of those with irradiation-induced leukemia, AML is observed.<sup>389</sup> Similarly, most HD patients who have developed a leukemic picture have been heavily irradiated.<sup>313,398</sup> It must be emphasized, however, that the development of leukemia after irradiation therapy is strongly influenced by the nature of the disease for which irradiation was given. The frequency of acute leukemia has been much higher in patients with polycythemia vera than in those with CLL even when both groups of patients received the same amount of <sup>32</sup>P.<sup>389</sup> In the reported cases of lymphosarcoma terminating in AML rather than ALL,<sup>336</sup> one suspects that radiation therapy may have been the cause, rather than natural evolution.

Although patients with rheumatic diseases may possibly be more likely to develop leukemia than the general population (page 1463), the observation of a dose-response relationship between x-ray therapy to the spine and the occurrence of leukemia in patients with ankylosing spondylitis (Table 46-4)

suggests that the x-ray therapy induced the leukemia. Local irradiation to the pelvic areas for carcinoma of the cervix<sup>350</sup> and <sup>131</sup>I irradiation of the thyroid<sup>393,400</sup> have not been associated with a significant increase in the frequency of leukemia, according to large-scale surveys. Thymic irradiation in children for enlarged thymus has been claimed to be associated with an increased frequency of leukemia,<sup>387,401</sup> but this has been disputed.<sup>335</sup> The reported cases are difficult to interpret since leukemia can occasionally begin with thymic enlargement (Chapter 54).

### *Leukemia in Radiologists*

Before the hazards of irradiation were recognized, death from leukemia was frequent among the pioneers in radiology.<sup>379</sup> With improved protective shielding the hazard has declined, but leukemia still is suggestively more frequent in radiologists than in other physicians (Table 46-4). Radiologists also have been found to acquire myeloma somewhat more frequently than do other physicians.<sup>372</sup>

### *Significance of Irradiation in the Overall Frequency of Leukemia*

If there is a "threshold" dose of irradiation below which there is no increase in frequency of leukemia, then irradiation is an interesting, but relatively insignificant, factor in the genesis of this disease. Conversely, if any excess of irradiation leads to an excess of leukemia, then attempts should be made to limit even minute amounts of irradiation exposure. Thus, studies of the relationship of small amounts of irradiation to the incidence of leukemia are of critical importance. Unfortunately, most studies have yielded equivocal results.

The incidence of the eventual development of leukemia in children whose mothers had diagnostic roentgenographic examinations while pregnant has been slightly, but seemingly significantly, more frequent than that in children whose mothers did not have these examinations during pregnancy.<sup>374</sup> (Table

46-4). Studies of the frequency of diagnostic irradiation procedures as related to the frequency of leukemia in patients so examined have yielded conflicting and, at best, equivocal data.<sup>108a,374</sup> It has been suggested that children exposed to low levels of irradiation can be divided into "susceptible" and "non-susceptible" groups as concerns irradiation leukemogenesis.<sup>310</sup> When large doses of radioactive agents, such as Thorotrast,<sup>391a</sup> were used for diagnostic purposes, a clearly increased incidence of AML and CML was observed. No relation between natural, background irradiation level, varying as much as nine-fold, and leukemia incidence was found in a survey made in Maine, New Hampshire, and Vermont.<sup>391</sup> Variability in extent of cosmic irradiation, as reflected by altitude of residence, bore no relation to frequency of leukemia.<sup>310</sup> With respect to the relation of "fallout" from nuclear explosions, the <sup>90</sup>strontium content of the bones of patients with leukemia was found to be less than normal,<sup>299</sup> perhaps reflecting changes in bone metabolism (Chapter 54). Most fallout from blasts in this country was concentrated in the Southwest, but so far no excess of leukemia has been noted in persons living in that region.<sup>74</sup>

Thus, the crucial question of a "threshold" relationship of irradiation and leukemia induction remains unanswered. Large doses of whole body irradiation (the A-bomb experience) and irradiation of leukemia-prone diseased populations (persons with polycythemia vera and perhaps other groups such as those with rheumatic diseases) are associated with an increased frequency of leukemia. The mechanism is unclear. However, if leukemia in man proves to be virus-related (page 1452), it is noteworthy that the time of onset of virus-related murine leukemia is accelerated by irradiation.<sup>250</sup> If the relation reflects an aberrant clone of cells derived from random induction of a chromosome abnormality in single cells by irradiation,<sup>311,369</sup> it is interesting that the Ph<sup>1</sup> chromosome abnormality has been reported as one of the abnormal, random findings in healthy, but irradiation-exposed, subjects (page 1441).

Unnecessary irradiation should certainly be avoided, but there is little to suggest that diagnostic, or even many therapeutic, irradiation procedures represent major factors in the induction of leukemia.

### Chemical Agents

As in persons with marrow aplasia and/or pancytopenia and in some with paroxysmal nocturnal hemoglobinuria (page 1463), AML has been observed in individuals who had been exposed to drugs alleged to produce marrow injury. Whether the resulting pancytopenia was an early manifestation of leukemia or whether the pancytopenia and the associated hematopoietic disturbance led to an increased chance of developing AML is moot. However, since certain congenital diseases of lymphatic tissue are associated with an increased incidence of lymphocytic leukemia and lymphoma (page 1463) rather than of myeloid leukemia, some support can be marshalled for the concept that a growth disorder of a specific hematopoietic tissue leads to an increase in frequency of the corresponding form of leukemia.

### Benzol

A variety of chemicals and drugs have been suggested as possible leukemogenic agents in human leukemia, but only benzol can be unequivocally implicated. Disturbances of the hematopoietic system, especially marrow aplasia with pancytopenia, in workers chronically exposed to benzol have been recognized for many years.<sup>319,377,380</sup> Of leukemia-like syndromes in such workers, Bernard and Braier found most to resemble AML.<sup>307</sup> The use of benzol in glues in the Italian shoe-manufacturing industry, which in large part was carried out in poorly ventilated private dwellings, forms the basis for the most complete data on leukemia following benzol exposure. The overwhelming predominance of AML or closely related syndromes, often preceded by periods of aplasia with pancytopenia, in such workers<sup>404</sup> provides compelling evidence for an etiologic relationship.

# Other Possible Leukemogenic Agents

As noted in Chapter 47, a period of pancytopenia with or without apparent marrow aplasia may precede the frank development of AML and related syndromes. Not only benzol but also other drugs that may cause pancytopenia have been suggested as causing AML. The rarity of case reports of chronic myelocytic or lymphocytic leukemia following exposure to such drugs, in contrast to the occurrence of AML, may be of significance. A few patients suffering from chloramphenicol-induced aplastic anemia have eventually developed AML.<sup>314</sup> Other drugs, such as hexachlorocyclohexane, have been suggested as leukemogens under similar clinical circumstances.<sup>356</sup> As noted (page 1463), the suggestion that the rheumatic diseases may be associated with an increased frequency of leukemia complicates the possible implication of phenylbutazone as a leukemogen. Phenylbutazone, which also may induce pancytopenia, has been suggestively associated with an increased frequency of AML in patients heavily treated with this drug.<sup>357</sup> The possible role of hydantoins in inducing HD is discussed in Chapter 50.

Various drugs used in cancer chemotherapy are leukemogenic in animals (Chapter 55) and it is possible that reports of acute leukemia developing in patients with myeloma,<sup>44</sup> HD,<sup>30</sup> or the cold agglutinin syndrome who had been treated with melphalan<sup>397</sup> imply such an effect.

# Miscellaneous Environmental Factors

A wide variety of environmental factors have been studied, often with conflicting findings.

Leukemia was more common in foreign-born Israeli children, especially those born in Africa, than in those born in Israel of foreign-born parents.<sup>144</sup> It was more common in both groups than in children born of parents born in Israel. The validity of the study is strengthened by the failure to show a similar association for lymphoma.

Leukemia has been reported to be less

common among the poor than among affluent groups.<sup>335</sup> However, other fairly comprehensive studies reported leukemia to be more common among the poor<sup>331</sup> and still other studies<sup>362</sup> found no relation between degree of affluence and frequency of leukemia. The last studies suggest that reports of lower frequency of leukemia in the poor may reflect less medical attention. Army inductees who developed HD were found to be from a higher economic class than those who did not develop the disease.<sup>416</sup>

Urban-rural differences in leukemia rates<sup>396</sup> can be explained, at least in part, by correction for the age of the population.<sup>380</sup> However, the finding of a higher urban than rural incidence in whites and the reverse in non-whites, and an increasing urban but decreasing rural incidence suggest that there may be true rural-urban differences.<sup>396</sup> In one study<sup>453</sup> a higher frequency of leukemia and myeloma, but not of lymphoma, was found in farmers as compared to non-farmers.

Suggestions have been made that appendectomy or tonsillectomy may predispose to the development of cancer, including leukemia and lymphoma,<sup>308,329,351,390</sup> but other studies have not substantiated such an association.<sup>340,347,358</sup>

The initial suggestion that vaccination with BCG might protect children from developing leukemia<sup>420</sup> was supported by one,<sup>391</sup> but not by a second,<sup>361</sup> large retrospective survey. Review of the incidence of leukemia and lymphoma revealed no differences between children randomly chosen to receive or not to receive BCG.<sup>418</sup>

No association of leukemia with trauma, penicillin usage, chronic infection,<sup>298</sup> influenza<sup>369</sup> or other viral infections<sup>406a</sup> in mothers, exchange transfusion in the neonatal period,<sup>321</sup> or drug history in general<sup>298</sup> was found. Children with leukemia were heavier at birth than those who did not develop leukemia,<sup>405a</sup> and certain dermatoglyphics were said to be uncommonly frequent in leukemic children.<sup>405b</sup> Greater frequency of atopic allergy in mothers of leukemic children and in leukemic children than in the general population has been claimed,<sup>378</sup> but no association

of atopy in children with leukemia was found in other studies<sup>329</sup> and a negative association also has been reported.<sup>327</sup> Whether smoking mothers do<sup>38,2</sup> or do not<sup>378</sup> have more offspring with leukemia than do non-smoking mothers is disputed. The ingestion of cold tablets has been suggested as a positive factor.<sup>379</sup> In one survey, birth order and maternal age were found to have no apparent influence,<sup>378</sup> while in another the frequency of leukemia was found to be greatest in the first-born child and in children born of older mothers.<sup>375</sup> A search for factors acting synergistically rather than as single agents revealed no increase in risk of childhood leukemia if (1) the mother had been irradiated before pregnancy, (2) had received diagnostic irradiation during pregnancy, or (3) had a history of "reproductive wastage"; or (4) the child had viral illness in early childhood.<sup>330</sup> However, when three or more of these factors were all present, the risk of leukemia was increased significantly. Leukemia has been reported in both husband and wife,<sup>300</sup> but surveys<sup>384,385</sup> suggest that such occurrences are coincidental. Psychologic factors have been considered important by some,<sup>376,371</sup> as has lightning.<sup>373</sup>

## Familial Disease

The evidence for an increased frequency of leukemia in relatives of affected patients remains in question, although more than 150 case reports of leukemia in two or more family members have appeared.<sup>124,465</sup> Miller<sup>177</sup> concluded that in siblings of leukemic children there was a four-fold increase in the risk of developing leukemia and in certain other surveys<sup>421,465</sup> a modest tendency for leukemia to be more common in relatives of patients with leukemia than in controls was observed. Others,<sup>468</sup> however, did not find this to be the case. Proper control groups for retrospective survey studies are difficult to design; the likelihood that a patient with leukemia or his parents will gain knowledge of such disease in other and distant family members is greater than would be that for control patients who are normal or suffering

from other diseases. The same statistical problems that complicate the interpretation of clustering of disease in time and space (page 1454) are applicable to isolated reports of multiple cases of disease in one family.

Perhaps part of the disagreement in the literature stems from combining all types of leukemia and related diseases rather than considering the diseases as separate entities. In many of the most impressive case reports of leukemia occurring in two or more family members, the type of leukemia was CLL, perhaps the most impressive report being that of a family in which six members developed CLL.<sup>452</sup> Gunz and Veale<sup>131</sup> found a slight excess of all types of leukemia in relatives of affected members, but considered the significance of this excess questionable for the reasons just discussed. However, using patients with CLL as indexes, they found a clear excess of CLL, CML, and perhaps lymphosarcoma and myeloma, but not of acute leukemia or other cancers in close relatives. No clear pattern of inheritance could be discerned. This survey did not include the families previously described by Gunz and associates<sup>432</sup> in which an apparent dominant pattern of inheritance was described. In another family, 9 of 29 members have an illness that resembles CML, but in some members the condition improved after splenectomy.<sup>461</sup>

Studies of concordance of leukemia in twins<sup>441,449,457,459</sup> usually, but not always,<sup>435</sup> have indicated a clear excess of acute leukemia in both twins when the twins were of the same sex. In cases concordant for leukemia,<sup>449</sup> ancillary evidence of homozygosity was found. In most reports, both twins developed the same type of acute leukemia during infancy or early childhood. No excess of leukemia was observed in non-twin siblings of children with leukemia<sup>439</sup> unless consanguinity was present in the family.<sup>443</sup> The latter finding suggests the possibility that a recessive gene that influences the development of leukemia may exist. Cases of concordance of reticulum cell sarcoma<sup>449,476</sup> or other types of lymphoma or leukemia<sup>457</sup> have been reported in twins, but the infrequency of these diseases in childhood leaves the sig-



nificance of the observations in doubt. Chromosome abnormalities were found to differ between a set of fraternal twins concordant for acute leukemia,<sup>466</sup> but were apparently the same in a set of identical twins concordant for AML in whom the defect was undetectable in nonleukemic cells.<sup>437</sup> Since identical twins have identical heredity it is tempting to ascribe their higher rate of concordant leukemia, as compared to that of fraternal twins, to inherited factors. Yet, it must be recognized that the in utero and postnatal environment is more likely to be the same in identical than in fraternal twins. Thus, twin data can be interpreted as supporting the concept that leukemia has an identifiable cause(s) rather than representing a completely random event in the population. They do little to identify the nature or timing of the cause.

Some familial instances of leukemia are ascribable to inheritance of a disease predisposing to leukemia (page 1462) such as ataxia telangiectasia<sup>472</sup> or Fanconi's aplastic anemia.<sup>411</sup> The possible relation of the inheritance of the Christchurch chromosome to CLL and of a Ph<sup>1</sup> chromosome to CML has been discussed (pages 1441-1444).

The finding of chromosome aberrations, including chromosome breaks and endoreduplication in healthy members of a family in which a child had partial D<sub>1</sub> trisomy and acute leukemia raised the question of some genetic factor influencing the chromosome pattern.<sup>479</sup>

With reference to genetic factors it is of some interest that the distribution of blood groups and transferrin types is quite similar in patients with leukemia as compared to the variations found in the general population.<sup>431</sup> Haptoglobin type 1/1 was found to be more frequent in patients with leukemia in general than in the general population,<sup>444</sup> although it was less frequent in patients with CLL.<sup>461</sup> Relatives of patients with CLL have not been found to have a high frequency of hypogammaglobulinemia,<sup>444</sup> in contrast to relatives of patients with Waldenström's macroglobulinemia.<sup>467</sup> Leukocyte HLA antigens were similar in patients with leukemia and in the gen-

eral population.<sup>442</sup> A higher frequency of certain HLA antigens has been reported in patients with HD<sup>418a,423</sup> but the significance of these observations is uncertain because of the differences in the reported findings.<sup>418a</sup> It is of some interest that genetic susceptibility to viral murine leukemia-lymphoma seems to be related to the frequency of certain murine transplantation antigens.<sup>266,448</sup>

## Ethnic Differences

A comparison of incidence in the white and non-white populations of the United States reveals that all forms of leukemia and lymphoma are more common in whites than in non-whites. These differences might be attributed to different standards of medical care were it not for the fact that myeloma appears to be more common in the non-white than in the white population (Table 46-2). Similarly, the apparent continuing rise in leukemia incidence in non-whites could be ascribed to improved medical care, but no such rise has been evident for HD (Table 46-2). These observations plus the lack of an early childhood peak of leukemia in non-whites<sup>421</sup> (Fig. 46-3) strongly suggest that ethnic differences play a role in the incidence of at least some of these diseases. Non-whites in the United States survey included Negro and Oriental populations.

The early peak of acute leukemia is less prominent in Japan than in the United States or in European Caucasian populations.<sup>40</sup> There are suggestions that lymphoblastic leukemia in Negro children is less responsive to therapy than that in Caucasians (Chapter 47). Observations also suggest that lymphoblastic leukemia may be more common in populations with European ancestry than in certain other populations.

Chronic lymphocytic leukemia is much less common in the Orient than in the United States. Comparison of mortality from leukemia of all varieties in the Japanese population, the white United States population, Japanese immigrants to the United States, and American-born Japanese indicates similar mortality rates in persons from ages 20 to

40.<sup>433</sup> Thereafter, all groups with Japanese ancestry show lower mortality rates than does the white population of the United States, even though Japanese living in the United States tend to have higher death rates than those living in Japan. Admitting that data for cell type are somewhat suspect, the difference appears to reflect a difference in the incidence of CLL primarily. Percentages of leukemia deaths listed as CLL were: Japan, 2.9; Japanese immigrants to the United States, 5.0; and United States whites, 31.5. These data could be interpreted as suggesting that the low frequency of CLL in Japanese is genetic rather than environmental, but certain cultural differences, such as diet, are not entirely erased by a change of residence. Lymphosarcoma in persons of all ages is less common in Japan than in the United States, but the lymphosarcoma mortality rates of Japanese immigrants to the United States more closely resemble those for the United States than for Japan.<sup>433</sup>

Some studies have provided evidence that there is an increased risk of leukemia in adults, but not children, of Russian Jewish heritage,<sup>430</sup> but others have failed to indicate a difference in leukemia rates in Jews versus non-Jews of similar country of origin.<sup>407</sup>

As greater attention is directed toward elucidating differences in the incidence of various diseases in ethnic groups and in different geographic areas, more definitive data are likely to be forthcoming.<sup>416</sup>

### Increased Frequency of Leukemia in Diseases Associated with Congenital Chromosome Abnormalities<sup>456</sup>

First reported in 1930,<sup>412</sup> the occurrence of leukemia in children with *Down's syndrome* (mongolism) has been documented by numerous case reports. In 1958, in a series of 677 children with acute leukemia, Stewart and coworkers<sup>470</sup> demonstrated that the number of those with Down's syndrome was 20

times greater than would be expected. Shortly thereafter, trisomy of chromosome 21 was demonstrated in children with Down's syndrome and was found to be a fairly constant defect in these subjects.<sup>456</sup> The leukemia in patients with Down's syndrome usually has been described as myeloblastic, but the frequency of lymphoblastic leukemia also has been reported to be increased.<sup>408,419,462</sup> A self-limited myeloblastic proliferation, indistinguishable from AML except for its disappearance without therapy, also has been noted in these children (Chapter 41),<sup>408,138</sup> as has polycythemia.<sup>475</sup> After leukemia develops the group G trisomy persists in the myeloid cells and the typical chromosome changes of acute leukemia (page 1443) often are superimposed.<sup>408a</sup> Of particular interest in this regard is a mongol in whom "congenital leukemia" was present; this remitted spontaneously, but then recurred. Chromosome abnormalities in addition to trisomy 21 were constantly present.<sup>438</sup> HD has developed in at least two patients with Down's syndrome<sup>151</sup> and there may be an increased frequency of other neoplasms as well.<sup>455</sup>

*Fanconi's anemia* appears to be associated with an abnormally high frequency of leukemia.<sup>420</sup> Chromosome breaks and gaps have been noted in high frequency in cells from patients with Fanconi's anemia, although there are no specific identifying defects and a diploid chromosome number has been present.<sup>474</sup> An increased frequency of acute leukemia also has been observed in such children.<sup>474</sup> Similar chromosome changes have been observed in *Bloom's syndrome*, which likewise is associated with increased frequency of leukemia.<sup>156</sup> The familial occurrence of the Ph<sup>1</sup> defect and its relation to CML have been discussed (page 1441), as has the possible relation of inherited G group abnormalities to familial cases of CLL (page 1444). A balanced group C-G translocation, found in three members of a family, was associated with ALL in an affected member whose mother, chromosome type unknown, also had ALL.<sup>120</sup> Patients with Klinefelter's syndrome who developed acute leukemia or lymphoma have been described.<sup>450</sup>

The significance of these chromosome abnormalities with respect to the development of leukemia is unknown.

## Association with Other Diseases

A variety of case reports as well as some patient surveys suggest that AML occurs with greater frequency in patients with paroxysmal nocturnal hemoglobinuria (PNH) (Chapter 29),<sup>413,439,440</sup> pernicious anemia (Chapter 15),<sup>410</sup> or aplastic anemia (Chapter 56), than in the general population and that AML and MM develop with some frequency in patients with sideroblastic anemia.<sup>415</sup> CML has also developed in a patient with PNH.<sup>472a</sup> All of these diseases are associated with abnormalities of cellular proliferation and one might postulate that in any disease characterized by changed proliferation there is an increased chance of producing a "leukemic clone." The absence of any known association of leukemia with disorders in which there is increased proliferation such as sickle cell anemia, thalassemia, or hereditary spherocytosis or in persons frequently donating blood weakens this interpretation. No association between hemolytic disease of the newborn (Chapter 27) and leukemia could be established in a retrospective survey.<sup>321</sup> Lymphoma may follow hyperthyroidism more often than would be expected.<sup>473</sup>

The frequency with which leukemia or lymphoma develops in association with congenital immune deficiency diseases<sup>422,426,429</sup> (Chapter 44) or in immunosuppressed patients<sup>163</sup> suggests that a fully functioning immune system provides some protection against these diseases.<sup>79</sup> Immune suppression is clearly a factor in inducing neoplasms in animals.<sup>417</sup> Alternatively, one could postulate that if intact portions of the immune system are overstimulated in persons with a congenital deficiency disease, the increased frequency of leukemia and lymphoma might merely reflect an increased probability of producing an abnormal clone of lymphoid cells.

The diffuse connective tissue disorders such

as rheumatoid arthritis, dermatomyositis, and lupus erythematosus appear to be associated with lymphocytic leukemia and lymphoma more often than can be accounted for by chance or in relation to therapy. Miller<sup>454</sup> reported observations in 14 cases patients and surveyed a large group of patients with cancer or lymphoid neoplasms for the presence of connective tissue disease. Such diseases were found in 1.9% of patients with lymphoid neoplasms, but in only 0.6% of patients with solid tumors of the breast, gastrointestinal tract and other tissues. Other surveys of their frequency in association with lymphoma range from 0.4<sup>409</sup> to 2.2%.<sup>404</sup> The incidence of these connective tissue diseases in the general population is estimated at 0.4%.<sup>451</sup> Lea and associates<sup>445</sup> surveyed a population of patients with leukemia and lymphoma for other diseases and compared them to a control population. Fractures, other injuries, and chronic sepsis were equally frequent in the populations studied, but rheumatic diseases were two to four times as frequent in patients with leukemia (type not specified), non-Hodgkin's lymphoma, and Hodgkin's disease than in the control population. The frequency of lymphoma also may be increased in Sjogren's syndrome, a disorder often associated with rheumatoid arthritis.<sup>472</sup>

There appears to be a negative association of leukemia with atherosclerosis<sup>460</sup> and essential hypertension.<sup>477</sup> Single case reports of leukemia developing in persons with conditions such as Gaucher's disease<sup>427</sup> and congenital agranulocytosis<sup>428</sup> are interesting, but of unknown significance.

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## The Acute Leukemias

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- Acute Myeloblastic Leukemia
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  - Clinical Differences between Morphologic Variants
- Other Forms of Acute Leukemia
  - Acute Eosinophilic Leukemia
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- Features Distinguishing ALL from AML
  - Age and Sex Distribution
  - Morphology
- Modes of Presentation
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- Course, Complications, and Cause of Death
- Survival
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  - Therapy of ALL
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THE acute leukemias are characterized by an increase in very immature leukocytes, usually myeloblasts or lymphoblasts, in the blood and/or marrow (Plates XVIII and XIX). Unless the subjects are treated, these diseases are fatal within a brief time. The lymphoblastic and myeloblastic forms differ in response to therapy, age distribution, and in various other ways, but there is considerable similarity in their modes of presentation

and complications. Acute leukemia can occur in persons of any age, from in utero (page 1476) even to age 100.<sup>19</sup> The history, possible etiologic factors, age and sex distribution, and the relation to other diseases have been discussed (Chapter 46).

### Acute Lymphoblastic Leukemia (ALL)

Although acute lymphoblastic leukemia can occur in individuals of any age, it is predominantly a disease of children. Typically, the leukemic cell is quite immature. It has fine nuclear chromatin and nucleoli. In the lymphoblast there is slight clumping of the chromatin, allowing the cell to be distinguished from a myeloblast (page 1478). Smaller, but still immature-appearing lymphocytic cells also are usually present. Rarely, especially in children, the cells appear rather mature but the clinical manifestations of ALL and chronic lymphocytic leukemia (Chapter 49) are so different that these two forms of leukemia would hardly be confused. (see ref 19). The only ALL variant of note is *lymphosarcoma cell leukemia* (leukosarcoma, lympholeukosarcoma) (Chapter 51). Although there may be morphologic<sup>148</sup> and perhaps biochemical<sup>163</sup> differences between this leukemia and typical ALL, in many instances the cells in either disease are morphologically indistinguishable from those in the other. We reserve the term "lymphosarcoma

cell leukemia" to designate cases diagnosed as lymphosarcoma (Chapter 51) at a time when the marrow and blood were not involved but which later became leukemic. In general, once conversion to a lymphoblastic picture occurs, the course is similar to that in other patients with ALL,<sup>148,167</sup> although the therapeutic response may not be as good as that in the patient in whom the ALL had a typical beginning. *Stem cell leukemia*, or "undifferentiated acute leukemia," is a term used by some investigators as a synonym for ALL.<sup>188</sup> Others have suggested that the survival rate may be lower in patients with "stem cell" than in those with "lymphoblastic" leukemia and claim that different clinical types of lymphoblastic leukemia can be distinguished morphologically.<sup>120</sup>

## Acute Myeloblastic Leukemia (AML)

The term "acute myeloblastic leukemia" (AML) will be used here to include a group of morphologically distinguishable syndromes (Table 47-1) which are similar in clinical presentation, course, and response to therapy. As judged by average survival time after the diagnosis has been made or by response to therapy, AML is probably the most malignant of all neoplastic diseases.

The exact frequency of the various subtypes of AML depends on definition since, in most instances, a completely "pure" picture is lacking.<sup>19,145</sup> Thus, in most patients with myeloblastic leukemia, in addition to the predominating myeloblasts, a few cells with lacy chromatin and folded nuclei, "monocytoid" myeloblasts, may be observed and a few "blasts" will have peroxidase-positive granules in the cytoplasm. Similarly, occasional proerythroblasts with megaloblastic features may be observed in the bone marrow.

### Morphologic Differences of AML Variants

The subtypes of AML listed in Table 47-1 are recognized by the characteristics of the

**Table 47-1. Diseases Included Under the General Term, Acute Myeloblastic Leukemia**

<i>Term</i>	<i>Characteristic Cell</i>
Acute myeloblastic leukemia (AML) ✓	Myeloblasts
Acute myelomonoblastic leukemia (Naegeli) (AMML)	Myeloblasts with some monocytic characteristics
Acute monoblastic leukemia (Schilling) (AMoL)	Immature monocytes
Acute promyelocytic leukemia (APML)	Promyelocytes
D <sub>1</sub> Guglielmo's syndromes	Myeloblasts—proerythroblasts
D <sub>2</sub> Guglielmo's disease	Proerythroblasts

abnormal cells. These are described below and are illustrated in Plates XVIII and XIX, pages 1474 and 1478.

**MYELOBLASTIC LEUKEMIA.** The predominant abnormal cells in blood and marrow are myeloblasts with round nuclei, fine nuclear chromatin, scanty cytoplasm, round and regular cytoplasmic borders, and no cytoplasmic granules. These cells are peroxidase negative.

**MYELOMONOBLASTIC LEUKEMIA (NÆGELI TYPE MONOCYTIC LEUKEMIA).** The most common abnormal cell in the blood and marrow has features of myeloblasts and of monocytes. The nucleus of many of the cells is folded and nuclear chromatin is fine but reticular. There often is more abundant cytoplasm than in myeloblasts and the cytoplasmic border may be irregular and may show pseudopod formation. Granules may or may not be present in the cytoplasm. In general, the cells in the blood tend to appear more monocytic than do those in the marrow.

**MONOBLASTIC LEUKEMIA (SCHILLING TYPE MONOCYTIC LEUKEMIA).** Virtually all abnormal cells have monocytic nuclear and cytoplasmic characteristics. The cells often are large and have serrated borders. Rarely, marked phagocytosis is evident as indicated by the presence of engulfed red corpuscles in the cytoplasm. The differences in morpho-



Fig. 47-1 Swollen and spongy gums in a patient with acute myelomonoblastic leukemia

logic appearance of "monoblasts" and myeloblasts as noted by light microscopy are even more evident on electron microscopy.<sup>61</sup> The monoblast nuclei exhibit extreme irregularity and are folded, while myeloblast nuclei are regular and round. Bridges between chromatin strands and "blebs" of the nuclear chromatin may explain the reticular, granulated appearance of the nuclear chromatin of monoblasts as seen by light microscopy. The Golgi apparatus of monoblasts is more prominent and cytoplasmic mitochondria, granules, vesicles, vacuoles, and pseudopods are more apparent in monoblasts than in myeloblasts or in myelomonoblasts.

**PROMYELOCYTIC LEUKEMIA.** More than half of the immature cells have cytoplasmic granulation which is peroxidase positive. The granules may be normal in appearance or abnormally large or oval, rather than round. Eosinophilic, basophilic, or both forms of granules may be present.<sup>88,152</sup> The nucleus may appear more immature than in the normal promyelocyte with very fine chromatin and prominent nucleoli.

**DI GUGLIELMO'S SYNDROMES (ACUTE AND CHRONIC ERYTHREMIC MYELOSIS, ERYTHROLEUKEMIA, see below).** In acute erythremic myelosis proerythroblasts predominate and there is little or no increase in myeloblasts. In chronic erythremic myelosis the erythroblasts are somewhat more mature. In eryth-

roleukemia there is also a marked increase in proerythroblasts but myeloblasts (or myelomonoblasts or promyelocytes) are increased as well. The proerythroblasts of Di Guglielmo's syndrome may resemble megaloblasts and manifest other morphologic abnormalities such as binucleation. In contrast to the negative PAS staining of other forms of leukemic myeloblasts and of normal proerythroblasts, the myeloblasts and proerythroblasts of Di Guglielmo's syndrome are PAS positive<sup>137</sup> (Plate XXII). Giant granules in eosinophils also have been described.<sup>58</sup>

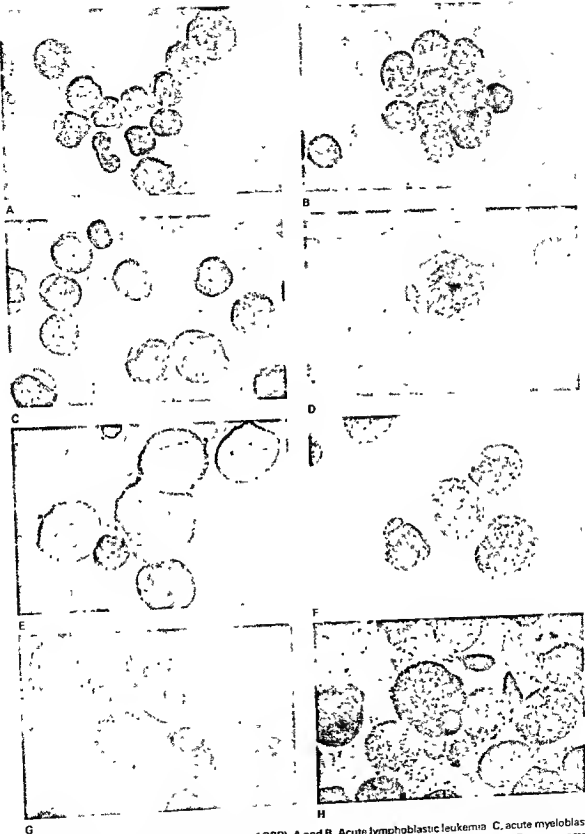
### Clinical Differences between Morphologic Variants

Features other than morphologic differences whereby the varieties of AML differ are few.<sup>19,20</sup>

**MYELOMONOBLASTIC AND MONOBLASTIC LEUKEMIA.** In myelomonoblastic or monoblastic leukemia, gum infiltration (Fig. 47-1) is a common feature; this is less frequent in other types of AL.<sup>19</sup> Increased frequency of skin infiltration in these varieties of AML has been claimed,<sup>59</sup> but this could not be confirmed in our series.<sup>19</sup> Some immature, leukemic monocytes migrate into inflammatory exudates while myeloblasts do not<sup>147</sup>; this migratory capability may explain the gum infiltration.<sup>116</sup> Whether monoblastic leukemia is a distinct entity or represents merely one end of the morphologic spectrum of AML is unknown at present. Much of the literature on this subject was written on the assumption that the "Nageli" type arose from the myeloblast while the "Schilling" variety arose from a different cell system. We now know that the myeloblast and monocyte have a common stem cell (Chapters 2 and 6).

**PROMYELOCYTIC LEUKEMIA.** In promyelocytic leukemia, particularly when large, abnormal-appearing granules are frequent in the cells, hypofibrinogenemic bleeding (Chapter 54) is more common than in other types of AML.<sup>19,43</sup> The suggestion that this may be a more severe disorder than other forms of AML<sup>11</sup> was not substantiated by analysis of our series of patients.<sup>20,43</sup>

# PLATE XVIII



*Varities of acute leukemia (Wright's stain,  $\times 1220$ ) A and B, Acute lymphoblastic leukemia. C, acute myeloblastic leukemia, D, acute myeloblastic leukemia, showing an Auer rod, E, acute myeloblastic leukemia. F is an example of the myelomonocytic (Naegeli) type of acute leukemia, G, acute monocytic leukemia of the Schilling variety. H, acute promyelocytic leukemia*

**DI GUGLIELMO'S SYNDROME.** The distinguished Italian hematologist, Di Guglielmo, first called attention to a condition characterized by neoplastic hyperplasia of both erythroblastic and leukoblastic tissues, which he called erythroleukemia. Shortly afterwards (1923), he applied the term acute erythremic myelosis to a disorder characterized by a generalized proliferation of the erythropoietic cells of the bone marrow, analogous to the leukocytic proliferation in leukemia.<sup>44</sup> He suggested that this is a primary and specific disease, an autonomous pathologic entity. The blood contains erythroblasts in all stages of maturation, but the most immature forms are found in disproportionately large numbers. Atypical forms and erythroblasts with multilobed nuclei and with altered nucleocytoplasmic ratios also are found. Reticulocytes are not usually increased in number. Increased levels of fetal hemoglobin may be found.<sup>34</sup> There is slight leukopenia, and occasional myelocytes and metamyelocytes, as well as reticuloendothelial cells, may be present in the blood smear. Platelets are decreased in number, often greatly. In contrast to the reduced proportion of erythropoietic elements characteristic of leukemia, these form the major part of the bone marrow cells, and basophilic forms predominate. At autopsy, infiltrations of primitive erythroblasts and reticuloendothelial cells are found not only in the hematopoietic organs but also in the kidneys, adrenal glands, and other tissues.

Subsequently Di Guglielmo called attention to a similar but more chronic disorder, chronic erythremic myelosis, also marked by the presence in the bone marrow of numerous erythroblasts but these were mainly the more mature forms. Hemorrhagic manifestations are less prominent. There may not be leukopenia and there is only moderate thrombocytopenia, but reticulocytes are usually increased, sometimes strikingly.

Acute erythemic myelosis and erythroleukemia follow a clinical course that cannot be distinguished from that of AML except for the excessive number of erythroblasts. These conditions respond to therapy as does AML.<sup>19</sup> As the name implies, chronic erythremic myelosis follows a more indolent

course and because most of the subjects eventually die of an AML-like disease<sup>41,100,172</sup> it may be best described as a form of "preleukemia" (page 1476). The nature of the anemia in Di Guglielmo's syndrome is discussed in Chapter 54 (page 1667). Distinguishing Di Guglielmo's syndrome from sideroblastic anemia and other idiopathic anemias with ineffective erythropoiesis (Chapter 18) may be difficult.<sup>172</sup> It has been suggested that whether or not idiopathic forms of anemia will eventuate in AML can be predicted by the normal appearance of the red cell precursors and normal platelet counts in the anemias that will not eventuate in AML in contrast to the presence of defects in heme synthesis in those that will.<sup>45</sup> Polycythemia vera has been reported to have terminated in acute erythremic myelosis.<sup>41</sup>

## Other Forms of Acute Leukemia

### Acute Eosinophilic Leukemia

In addition to acute leukemia in which eosinophilic promyelocytes are the predominant cells and the leukemia that might be considered chronic eosinophilic leukemia (Chapter 48) (page 1513),<sup>88,152</sup> there is another syndrome that may be termed "eosinophilic leukemia." In 1919, Griffin<sup>73</sup> described a patient with hepatosplenomegaly, lymphadenopathy, and shifting pulmonary infiltrates who died with severe congestive heart failure. This patient had as many as  $170 \times 10^9$  eosinophils/l of blood, most of which were segmented and abnormally large but had sparse granulation. He was anemic and, at postmortem examination, endocarditis and eosinophilic infiltration of organs such as the spleen and lymph nodes were noted. Numerous reports of similar cases have appeared under diverse headings such as "eosinophilic leukemia,"<sup>9,10,78</sup> "disseminated eosinophilic collagen disease,"<sup>160</sup> and "Löfller's endocarditis parietalis fibroplastica with eosinophilia."<sup>182</sup> The similarity of the course and complications of a number of the cases suggests that the disease is a distinct entity and whether it should be called leukemia or given



some other name seems immaterial in our present state of ignorance regarding the cause of the eosinophilia. Anemia, neutropenia, and thrombocytopenia may or may not accompany the eosinophilia. Pulmonary symptoms associated with shifting infiltrates, sterile endocarditis with congestive heart failure, and migratory thrombophlebitis with embolization characterize the course of the disease and constitute the usual causes of death. A variety of standards for a diagnosis of true "leukemia" have been proposed<sup>7,78,160</sup> including a terminal blastic stage.<sup>162</sup> Blastic transformation of the disease has been noted by us and by others,<sup>162</sup> suggesting that the designation of leukemia is appropriate in at least some patients. However, marked eosinophilia as such must not be interpreted automatically as eosinophilic leukemia. Unless there are ancillary findings suggesting that the patient will follow the course described in acute or chronic (Chapter 48) eosinophilic leukemia it is probably unwise to use this term. Such patients may prove to have periarthritis, carcinoma, Hodgkin's disease, or a variety of other diseases associated with eosinophilia (Chapter 41), or eosinophilia may persist for many years and remain unexplained.

### Mast Cell Leukemia

In the congenital disease, "urticaria pigmentosa" there is an associated increase in concentration of mast cells in the ultraviolet-sensitive skin of the patients. An acquired disease in adults has similar characteristics. In some patients with either congenital or acquired disease, increased mast cells also are found in the liver and other organs; in such cases the designation "systemic mastocytosis" has been applied.<sup>30</sup> In extreme cases of systemic mastocytosis, large numbers of mast cells are found in the patient's blood and the condition may prove fatal, leading some authors to designate it as "mast cell leukemia."<sup>67</sup> In the sense that the mast cell accumulation does not seem to represent a response to an identified stimulus, the disease can be considered a tumor and, when the number of mast cells in the blood is high,

leukemia. Since the relationship of the mast cell to basophils or other myeloid elements is poorly defined (Chapter 2), the syndrome cannot be meaningfully related to hematopoietic tumors at the present time. A chronic form of leukemia may be associated with a marked increase in basophils (Chapter 48), and, as noted (page 1474), in promyelocytic leukemia prominent basophilic granules may be present in the immature cells.

### Congenital Leukemia

More than 100 cases of congenital leukemia in which evidence of the disease was present within the first week of life have been reported.<sup>19,25,71,15,83,166</sup> Most of these newborn infants as well as most of those developing leukemia during the first year of life have suffered from AML rather than ALL, in contrast to the usual findings in children developing leukemia after the first year (page 1478). Congenital leukemia has two features that are uncommon in older patients with AML—the patients frequently are not anemic and the majority have leukemic infiltration of the skin. *Leukemoid reactions* of unknown cause closely mimicking AML also have been reported in newborns and must be distinguished from true AML.<sup>4,51,126</sup> As with AML manifested at any age, in congenital leukemia the survival usually is of short duration although spontaneous<sup>36</sup> and chemotherapy-induced<sup>136</sup> remissions have been reported. One mother delivered at least two and probably four children with congenital leukemia.<sup>29</sup> Since there was no other family history of leukemia and chromosome studies showed no abnormality in either parent, some in utero event leading to such a remarkable occurrence might be postulated. However, an abnormal uterine environment could not account for the observation that of two pairs of twins—one fraternal<sup>89</sup> and one identical<sup>180</sup>—only one of each pair was affected by congenital leukemia.

### Preleukemia<sup>17</sup>

Diseases which may terminate in AML were discussed in Chapter 46. In addition, a

hematologic defect, most often anemia, may be noted in patients in whom no diagnosis can be established after careful study. Some months or even years later a fairly typical picture of AML evolves. "Preleukemia"<sup>17</sup> seems a reasonable term for designation of this condition and has been applied when the diagnosis of AML may be suspected but cannot be made with any confidence. The term "smoldering leukemia"<sup>133</sup> also has been used to denote this condition but it implies a predicted behavior for the disease and the leukemia may not "smolder" once it becomes apparent.<sup>19</sup> Certain cases described as chronic monocytic leukemia<sup>157</sup> also can fit the designation of preleukemia as used here. Most patients with preleukemia have been elderly, but this syndrome can occur in persons of any age, including infants.<sup>47</sup> Most patients with preleukemia have developed AML, but ALL has also been reported to follow preleukemia in children.<sup>123</sup> Approximately 15% of our patients with AML have undergone a preleukemic phase. Except for the diagnostic enigma when these patients were first examined, the eventual symptoms and course did not differ significantly from those experienced by other patients with AML. Survival from the time of diagnosis to death was also similar to that of other patients with AML.<sup>19</sup>

The most consistent abnormality identified in patients who prove to have preleukemia is the presence of anemia.<sup>16,17,19,122</sup> The anemia usually is normocytic and normochromic and is accompanied by a normal or decreased reticulocyte count. Sometimes it is microcytic and hypochromic, and in such instances ringed sideroblasts (Chapter 47) often are demonstrable in the bone marrow. In other cases a hypochromic cell with basophilic stippling is seen beside normocytic or macrocytic normochromic red cells. In the presence of macrocytic anemia and cells in the bone marrow which suggest megaloblasts, a diagnosis of Di Guglielmo's syndrome has often been entertained when various causes of megaloblastosis (Chapter 14) have been ruled out. Blood neutrophils may be normal or decreased in number and the number of platelets may be normal, decreased, or even increased. The percentage of myeloblasts in

the marrow may be normal or slightly increased. There is no exact percentage of myeloblasts that "establishes" a diagnosis of AML, but we would be very reluctant to make a firm diagnosis if fewer than 10% were present. The spleen and/or liver may be palpably enlarged, but sternal tenderness and lymphadenopathy are unusual in preleukemia. Although no test is available to determine what patients with unexplained anemia or with a reduction in two or all the formed elements of the blood ("bi-" or pancytopenia) will develop AML, certain findings suggest this diagnosis. Thus, the presence of an aneuploid cell line in direct chromosome preparations from bone marrow is strong evidence for leukemia<sup>127</sup> (Chapter 46). Many patients with AML have an abnormally low leukocyte alkaline phosphatase; such a finding suggests this diagnosis or paroxysmal nocturnal hemoglobinuria (Chapter 46). *In vitro* culture, in semisolid media, of marrow from some preleukemic patients may be helpful.<sup>72</sup> No growth may appear or abnormally small colonies may form. Marrow cells from such patients may also fail to mature in liquid culture.<sup>69a</sup>

### Miscellaneous Forms

Chloroma is a term applied to localized tumors of granulocytic tissue, especially those involving the orbit.<sup>49,143</sup> The name is derived from the observation that the cut surface of such tumors transiently turns green when exposed to light, a phenomenon that probably reflects the myeloperoxidase content of the cellular lysosomes.<sup>149</sup> On rare occasions, chloromas may appear at a time when the leukemia is in an early stage, but no convincing reports<sup>179</sup> of cure of an isolated chloroma have appeared since 1927.<sup>111</sup> Certain authors<sup>130,139</sup> have considered the leukemia associated with chloromas to differ from other forms of AML.

Still other categories of AML have been suggested<sup>120</sup>; *megakaryocytic leukemia*<sup>3</sup> is discussed in Chapter 48. Occasionally myeloblasts are smaller than normal; in such cases the term *micromyeloblastic leukemia* has been applied. An occasional patient maintains

a normal or even increased number of apparently mature segmented neutrophils despite the increase in blasts in the marrow, a condition which has been designated acute neutrophilic leukemia.<sup>46</sup> The term *subacute* has been applied in two situations—for AML in patients who have lived for more than six months and in patients in whom some neutrophilic maturation was present.<sup>19</sup>

The malignant *reticulosos* (Chapter 51) have some features suggesting acute leukemia.

## Distinguishing ALL from AML

### Age and Sex Distributions

The age distributions of ALL and AML differ quite markedly (Fig. 47-2). More than 80% of cases of leukemia in children were lymphoblastic in origin and in certain other studies.<sup>19,42,65,118</sup> However, 32% of patients seen at the Children's Cancer Foundation in Boston were considered to have AML,<sup>60</sup> and AML was thought to occur almost as frequently as ALL in children in one British series.<sup>140</sup> In persons in the teens and early twenties, ALL and AML occur with about equal frequency, but, as age progresses, AML becomes increasingly predominant whereas ALL is a relatively rare disease in persons 45 years of age or older. Sex ratios are approximately 3 males to 2 females for both conditions (Chapter 46).

Morphology (Plates XVIII and XIX, pages 1474 and 1478)

Although there are certain symptoms, signs, and laboratory features which in general are more common in ALL than in AML, in individual cases the differentiation depends on examination of the immature cells.<sup>19</sup> The skilled morphologist usually can make the

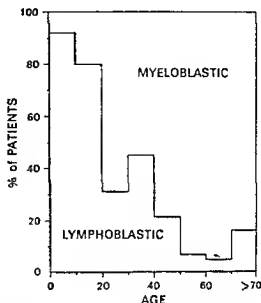


Fig. 47-2. Relative frequencies of ALL and AML as they vary with age in 500 patients; two series combined.<sup>19,65</sup>

differentiation with some certainty by examining well-made blood or bone marrow smears treated with Romanowsky stains. When slides properly prepared with Romanowsky's stain have been subjected to blind review by a panel of expert morphologists, general agreement as to type has varied from 75 to 95%.<sup>80,112,178</sup> (see also ref. 19). The diagnosis of AML is obvious when, together with "blasts" containing nucleoli, cells are present with evident granules, which are confirmed as peroxidase positive, or cells with monocytoid features are found, or when abnormal erythroblasts suggesting Di Guglielmo's syndrome are evident. Auer rods (Chapter 46) are easily demonstrable in "blasts" in approximately 10% of patients with AML<sup>140</sup> and can be found in an even higher percentage when a careful search is made.<sup>19</sup> In contrast, in many ALL patients lymphoblastic maturation has proceeded to the

## PLATE XIX

Acute leukemia, various forms (blood smears,  $\times 1000$ )

A, B, C, O. Acute lymphoblastic leukemia. A and B, Wright's stain. C, peroxidase stain (note the negative reaction and compare with the positive reaction in cases of AML and AMML shown in Plate XX, C and D). D, PAS stain (note the coarse granulation in a few cells)

E, F, Acute myeloblastic leukemia, Wright's stain. Auer bodies are present in F and in one cell in E

G, Acute promyelocytic leukemia

H, I, Acute monocytic leukemia

# PLATE XIX



A



B



C



D



E



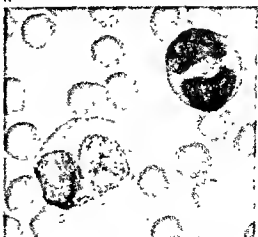
F



G



H



I

point that the nuclear chromatin is easily seen as being more dense than in myeloblasts and may be identified as lymphocytic. However, in other patients, certainly less than half, the predominant cell is a blast having few obvious signs of cytoplasmic or nuclear maturation. Adding to the difficulty, occasional promyelocytes or myelocytes may be observed in the blood of patients with ALL, especially in those with high leukocyte counts.<sup>19,125</sup> Thus, identification of blasts "by the company they keep" is not wholly reliable and may account for some reports of "mixed" leukemia.<sup>37</sup> In this circumstance, more subtle morphologic features must be looked for. The nuclear chromatin of lymphoblasts is slightly but visibly clumped in an irregular pattern, while that of the myeloblast is uniformly fine, finely granular, or lacelike in appearance. The nuclear membrane is of irregular thickness in ALL and thin and of uniform thickness in AML, a feature best appreciated in living cells examined by means of phase microscopy. There tends to be a larger number of nucleoli in myeloblasts than in lymphoblasts (Chapter 6).

Stains for peroxidase, periodic acid-Schiff staining, and Sudan Black B staining will sometimes help to distinguish AML from ALL.<sup>80,81</sup> However, these detect maturing characteristics of cytoplasm, granules in AML, or glycogen in ALL and usually confirm what is seen in Romanowsky stained smears. They are of limited help in the difficult case in which the cells are uniformly immature. Leukocyte alkaline phosphatase (LAP) stains are of some help if enough mature neutrophils are available to permit a meaningful test. The LAP score tends to be high in ALL and low in AML.<sup>81</sup> Finding elevated serum or urinary muramidase often identifies acute monoblastic or myelomonoblastic leukemia,<sup>33,153</sup> but such cases usually are readily recognized on morphologic study. A variety of other tests of cellular function or chemistry may help to distinguish ALL from AML, such as motility (Chapter 6), arylsulphatase staining,<sup>108,121</sup> and esterase staining with naphthyl acetate.<sup>144</sup> However, these tests are not generally available and have not been proven to be exact enough to justify their

routine use. Serum B<sub>12</sub> levels may be elevated in AML but are usually normal in ALL.<sup>8</sup>

Study of chromosome changes in direct marrow or blood preparations may be helpful. When aneuploidy is present the line is almost invariably hyperdiploid in ALL, whereas there may be either hyper- or hypoploidy in AML (Chapter 46).

## Modes of Presentation<sup>19,140,159</sup>

### Symptoms

The most common complaint at the time of diagnosis is that of *fatigue* or *malaise*, or of a poorly defined sense of ill health (Table 47-2). In the average patient the complaint has been present for several months before the diagnosis is made. The symptomatic period preceding diagnosis tends to be longer in AML than in ALL patients, perhaps reflecting the frequency of "preleukemia" (page 1477) in the former. The presence and severity of fatigue bear some relation to the presence and severity of anemia.<sup>19</sup> Fatigue is the primary complaint of about half of the patients and is part of the presenting picture in most.

*Fever*, with or without demonstrable infection or other symptoms of infection, is the primary presenting complaint in 15 to 20% of patients. Fever has been noted by approximately half of the patients with AML by the time the diagnosis has been made and is even more common in patients with ALL. A documented infection is less common than a history of fever. Complaints of sweating, usually night sweats, are rare in the absence of fever. As noted in Chapter 54, severe neutropenia usually is present in the infected patient, but there are no demonstrable laboratory features that correlate with fever without infection.<sup>18</sup>

Approximately 10% of patients consult a physician because of easy bruising, petechiae, nosebleed, or other hemorrhagic phenomena (Figs. 47-3 and 47-4); half have noted such difficulties by the time of diagnosis. The presence and severity of complaints of hemorrhagic phenomena correlate well with the severity of thrombocytopenia (Chapter 54).

Table 47-2. Symptoms and Signs at Time of Diagnosis of Acute Leukemia

	ALL (178 Patients) (% of Patients)	AML (144 Patients) (% of Patients)
Symptoms		
Fatigue	92	86
Fever without infection	71	48
Infection	17	26
Purpura	51	30
Other hemorrhage	27	34
Bone or joint pain	79	20
Weight loss	66	47
Abnormal masses	62	11
Physical findings		
Splenomegaly	86✓	60
Hepatomegaly	✓74	54
Lymphadenopathy	76	47
Sternal tenderness	69	65
Petechias and/or ecchymoses	50	46
Fundic hemorrhage	14	16

*Weight loss* is noted by about one half the patients but is rarely severe and thus is rarely the chief complaint. Pain in the bones or joints occasionally is severe enough to bring the patient to the physician and is more common in ALL than in AML patients. Head-

ache or cough without apparent pulmonary infection is experienced with some frequency but is rarely a primary feature.

The patient with AML rarely notes enlarged nodes or abdominal masses, but the ALL patient does so more frequently. Since

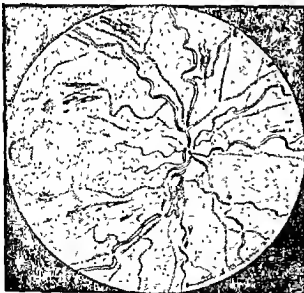


Fig 47-3 Fundus oculi in a patient with acute leukemia. There are numerous flame-shaped hemorrhages as well as small and large irregularly round hemorrhages. Clear centers are visible in some of the hemorrhages. The caliber of the veins is greatly increased, that of the arteries decreased. (Courtesy of Dr. Alan C. Woods)

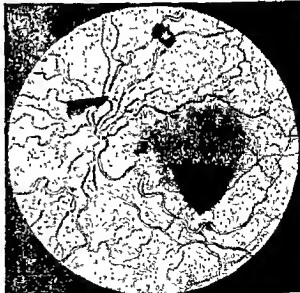


Fig 47-4 Fundus oculi in a patient with acute lymphoblastic leukemia. The outlines of the optic disk are blurred. There are striae and subhyaloid hemorrhages. The caliber of the veins is increased, that of the arteries decreased. The vessels are tortuous and many are marked by whitish streaks. The retina has a veil-like appearance. (Courtesy of Dr. Alan C. Woods)

virtually any organ can be involved by leukemic infiltration or by hemorrhage or infection, a variety of unusual presenting complaints such as seizures, loss of vision, and gum hypertrophy are occasionally noted.

#### Physical and Radiologic Findings at Time of Diagnosis (Table 47-2)<sup>19</sup>

The primary physical findings relate to anemia (*pallor*, tachycardia, cardiac murmurs presumably of hemic origin), thrombocytopenia (petechiae and ecchymoses or other hemorrhages), neutropenia (infection), or leukemic infiltration (splenomegaly, hepatomegaly, lymphadenopathy, sternal tenderness).

Slightly more than half the patients with AML have *splenomegaly* and/or *hepatomegaly* at the time of diagnosis. The spleen, if palpable, usually is less than 5 cm below the costal margin. It is more frequently palpable and tends to be larger in patients with ALL than in AML.<sup>12,19</sup> *Lymph nodes* may be infiltrated and enlarged in AML patients, but are less prominent than in those with ALL.<sup>12,19</sup> Hilar lymph node enlargement or thymic enlargement is found on chest x ray in a small percentage of patients with ALL, but is unusual in those with AML. Parenchymal pulmonary infiltration is uncommon in acute leukemia (Chapter 54). Hepatomegaly rarely is symptomatic; neither is splenomegaly, unless a splenic infarct occurs. The course of a splenic infarct, recognized by left upper-quadrant pain accentuated by respiration and accompanied by friction rub, is usually uneventful (Chapter 54). Fewer than 10% of patients suffer recognized splenic infarcts during the course of disease.

*Sternal tenderness* is demonstrable in approximately two thirds of the patients and thus is a fairly reliable sign although it is not quite as common as it is in CML (Chapter 48). The usefulness of sternal tenderness as a diagnostic sign has been questioned since "pain threshold" as measured by applying graded pressure to one spot on the sternum was not "reliably decreased" in leukemia.<sup>121</sup> Sternal tenderness, however, usually does not

involve the entire sternum. Rather, as firm pressure is sequentially and systematically applied from top to bottom of the sternum, a small area, most commonly in the mid-body, is found which is quite tender. Normal persons may say that such pressure is "painful" if questioned, but in them spontaneous complaints or withdrawal with wincing during the examination are not usually elicited. Sternal tenderness also can be found in other patients such as those with severe hemolytic anemia and very hyperplastic bone marrow, and thus is not limited to leukemia patients.

Just as complaints of bone pain are more common in ALL than in AML patients, so are *bone lesions* more commonly demonstrable by x ray in ALL than in AML patients<sup>12,69,176</sup> (Chapter 54). The *skin* is noticeably infiltrated in approximately 10% of leukemia patients. This usually takes the form of a diffuse, plaque-like, violaceous lesion (Chapter 54). Gum infiltration, as previously noted, is suggestive of myelomonoblastic leukemia (Fig. 47-1). Physical evidence of disease at the time of diagnosis is lacking in a small but significant number of patients with AML but this is most unusual in ALL patients.

*Meningeal infiltration* may be present at the time of diagnosis<sup>93</sup> and lumbar puncture reveals leukemic cells in the CSF in some asymptomatic patients (Chapter 54). The frequency and significance of all of the above complications are discussed further in Chapter 54.

#### Laboratory Findings

*Blood findings* at the time of diagnosis are presented in Table 47-3.

The *leukocyte count* is elevated in slightly more than half of the patients. Even when the count is normal or low, blasts usually are demonstrable in the blood smear ("sub-leukemic leukemia"). More than  $100 \times 10^9$  leukocytes/l are present in fewer than 20% of patients, although more than  $1,000 \times 10^9/l$  may be found rarely. Leukocyte counts generally mirror blast levels since neutro-

Table 47-3. The Blood at Diagnosis of Acute Leukemia

	ALL (178 Patients) (%)	AML (144 Patients) (%)
Total leukocytes (per liter)		
$< 5.0 \times 10^9$	25	29
$5.0-10.0 \times 10^9$	15	15
$10.0-49.0 \times 10^9$	34	28
$50.0-99.0 \times 10^9$	15	13
$> 100.0 \times 10^9$	11	15
Neutrophils (per liter)		
$< 1.0 \times 10^9$	73	49
$1.0-2.0 \times 10^9$	9	16
$> 2.0 \times 10^9$	18	35
WBC (/ $\mu$ l)		
$< 0.30$	65	60
$> 0.30$ < normal	33	37
normal	2	3
Reticulocytes (>3%)	6	16
Nucleated red cells noted	25	45
Platelets (per liter)		
$< 50 \times 10^9$	62	39
$50-150 \times 10^9$	30	44
$> 150 \times 10^9$	8	17

penia is often present. In general, there is little correlation between the number of blasts in the blood and the size of the spleen, or between blast levels and other manifestations of infiltration.<sup>19,101,125</sup>

The frequency of *aleukemic leukemia*, which we define as no blasts seen on blood smear, depends on the intensity of the search. If routine differential counting is employed, no blasts will be noted in approximately 5% of patients, but scanning of smears or examination of buffy-coat preparations reduces this figure. Aleukemic patients usually are leukopenic, thus providing a clue to a leukocyte abnormality. ✓

*Anemia* is an important finding and often is quite severe. It is usually normocytic and normochromic, although in AML with *Di Guglielmo* features it may be macrocytic. Nucleated red cells may be noted in the blood and are more common in AML than in ALL patients (Table 47-3). Reticulocytopenia usually is present (Table 47-3), reflecting decreased cell production (Chapter 54).

*Thrombocytopenia* is present in most instances and is frequently pronounced at diagnosis. On very rare occasions thrombocytosis may be present.<sup>5</sup> Large bizarre platelets similar to those seen in CML (Chapter 48) or polycythemia vera (Chapter 30) may be observed in AML.

*Serum uric acid* levels are elevated in approximately half the patients, and urinary uric acid excretion is increased in almost all.<sup>87</sup> Symptomatic gout is quite rare<sup>181</sup> (Chapter 54). Uric acid nephropathy (Chapter 54) occasionally is observed in untreated patients and constitutes a significant hazard in patients receiving treatment unless appropriate preventive measures are taken<sup>103</sup> (Chapter 54). Hypercalcemia has been observed but is uncommon (Chapter 54). *Serum protein*<sup>53</sup> changes are somewhat different in ALL than in AML patients in that diffuse hypergammaglobulinemia is common in those with AML whereas gamma globulin usually is normal in those with ALL. Serum albumin is normal in most patients at the time of diagnosis, but declines as disease advances. Elevation of beta globulins is common and increased alpha globulins often reflect the presence of fever or infection (Chapter 54). On rare occasions, monoclonal protein spikes may be present.<sup>161</sup> Although decreased serum gamma globulin is unusual, anergy may be present.<sup>85</sup>

*Bone marrow* biopsy most commonly reveals a hypercellular marrow, but may reveal hypocellular or even (very rarely) necrotic parenchyma.<sup>24</sup> Thus it is to be anticipated that marrow aspiration usually will yield adequate material for examination and for diagnosis. In most patients the predominant cell in marrow smears is a blast. However, in some patients, particularly those with AML, replacement by blasts is only moderate and numerous normally appearing marrow cells may be present. Diagnostic marrow examination is unnecessary if numerous blasts are present in the blood. Marrow examination may be quite important in distinguishing between active disease and drug toxicity as a cause of cytopenia if no blasts are demonstrable on the blood smear. Because blasts



invariably disappear from the blood before disappearing from the marrow, no advantage is gained by study of the marrow in evaluating the course of therapy as long as blasts are present in the blood.

## Differential Diagnosis

There are but a few potentially benign conditions that mimic acute leukemia. Most diagnostic difficulties or delayed diagnoses represent failure to consider the diagnosis or to make adequate examinations. In addition to the dilemma posed by the patient with preleukemia, patients with CML who present in blast crisis (Chapter 48) and patients with myelofibrosis who have increased myeloblasts in the marrow or even in the blood (Chapter 57) must be differentiated. As reviewed in Chapters 48 and 57, other features suggesting those diseases often allow the correct diagnosis to be made.

Patients recovering from neutropenia (Chapter 41) or aplastic anemia (Chapter 56) may have a marrow in which myeloblasts and promyelocytes predominate if the specimen is obtained when neutrophil production is recovering. For this reason the patient who appears to have "aleukemic leukemia" (page 1482) should be observed for a short time to be certain that the blastic marrow does not mature or that neutrophils do not return to the blood before a firm diagnosis of leukemia is made, unless other features also suggest the diagnosis. We have observed one 17 year old boy, who has been neutropenic since birth, in whom repeated bone marrow aspirates contain 30 to 45% monocytoid myeloblasts and promyelocytes but virtually no myelocytes or more mature cells. It only has been the knowledge of prolonged neutropenia and the absence of anemia or thrombocytopenia that has allowed us to exclude a diagnosis of AML.

Miliary tuberculosis has been reported to produce a leukemoid reaction indistinguishable from AML (Chapter 41), even with Auer rods in the myeloblasts.<sup>110</sup> However, we have seen no reports of successful antituberculosis therapy which induced hematopoietic recov-

ery in such cases. The patients may well have had AML as well as tuberculosis rather than having a leukemoid reaction.

Tumor cells in bone marrow, especially in neuroblastoma,<sup>67</sup> may be confused with lymphoblasts (Plate XXIV). These cells tend to occur in clumps while leukemic blasts are fairly uniformly distributed in marrow smears or biopsy specimens. The cells of infectious mononucleosis (Chapter 43) should not be confused with lymphoblasts by the skilled morphologist.

Other conditions in which myeloblasts and promyelocytes have been observed in the blood include severe pyogenic infections, acute hemolytic anemia, metastatic tumors, dehydration, and juvenile rheumatoid arthritis.<sup>92</sup> This type of leukemoid reaction (Chapter 41) seems more common in infants and young children than in adults and the blood usually suggests CML rather than AML.

## Course, Complications, and Cause of Death

The course of acute leukemia may be favorably or adversely affected by therapy. The chief causes of 90% of deaths and of much of the morbidity reflect two of the common presenting complaints—hemorrhage due to thrombocytopenia and infection due to neutropenia (Table 47-4).<sup>19,109</sup> Fatal hemorrhages in AML are usually subarachnoid, but a significant number of patients die from gastrointestinal hemorrhage or from intracerebral hemorrhage associated with a rapidly rising white blood cell count and leukostatic cerebral lesions (Chapter 54). Leukostatic cerebral lesions are more often found in AML than in ALL patients, while fatal gastrointestinal hemorrhage is more common in those with ALL than in AML.<sup>19,84</sup> Occasionally, fatal hemorrhage into bronchi, pericardium, or peritoneum is encountered. Liberal use of platelet transfusions (Chapter 54) reduces the frequency of fatal hemorrhage in acute leukemia. The proportion of patients at the National Cancer Institute dying of hemorrhage decreased, while the proportion

dying of infection increased over a 10-year period (Table 47-4),<sup>84</sup> a change attributed to the increased use of platelet transfusions. In this study the type of fatal infection changed during the same period; staphylococcal infections decreased and fungal infections increased, a change presumably attributable to changed antibiotic usage (Chapter 54).

Other demonstrable causes of death include leukemic infiltration producing hepatic, pulmonary, cardiac, or renal failure, as well as meningeal lesions (Chapter 54).

The primary causes of death are also the primary complications and causes of morbidity during life (see Chapter 54). However, since some infiltrating lesions which are life-threatening, such as *meningeal infiltration*, can be corrected by local therapy (Chapter 54), these constitute more common causes of morbidity than mortality in properly managed patients. Meningeal infiltration is much more frequent in ALL than in AML. This perhaps simply reflects longer survival in ALL for, as discussed in Chapter 54, such infiltration may occur during periods of hematologic remission as well as with active disease.

Anemia, with its concomitant symptoms and signs, must be combatted by judicious use of transfusions in almost all patients with active disease. Fever, in the absence of infection, may be accompanied by severe or by minor symptoms (Chapter 54). Complicating infection is common during any period of active disease<sup>45</sup> (Chapter 54) and can develop even during remission if maintenance therapy is sufficiently intense to be immunosuppres-

sive.<sup>22,131</sup> Early recognition and prompt, specific antimicrobial chemotherapy are necessary if infections in a leukemia patient are to be controlled (Chapter 54). It was suggested that adjunctive therapy, that directed toward treating complications rather than toward systemic control of leukemia, prolonged life before effective chemotherapy was introduced.<sup>14</sup>

## Survival

The duration of life after diagnosis of AML with or without therapy is quite variable. Survival curves for three series of patients treated by us are shown in Figure 47-5B. Patients treated before effective therapy was available lived an average of 2.5 months, many dying very shortly after diagnosis; only rarely did patients live more than a year. These results were similar to those in comparable series.<sup>65</sup> Survival in patients who were treated with 6-MP but failed to achieve remission was virtually identical to that of patients given no effective therapy. In those in whom remission was induced by therapy, life was prolonged. With modern combined drug therapy (page 1494) the percentage of AML patients achieving complete remissions has increased<sup>7a,36a,40,141</sup> and some have survived two years or longer.<sup>7a</sup> However, as many as 25<sup>36a</sup> to 32<sup>7a</sup> % have died in four to six weeks because of marrow hypoplasia induced by therapy or due to the disease itself.

Many factors influence survival, including the drugs used, patient age, facilities avail-

**Table 47-4. Changing Causes of Death in Acute Leukemia at the National Cancer Institute**

Cause of Death	Total Group (366 Patients)	1954-1959 (124 Patients)	1960-1963 (131 Patients)
Infection	38%	27%	48%*
Hemorrhage	21%	27%	14%*
Infection and hemorrhage	32%	40%	24%*
Other	6%	5%	7%
Obscure	4%	2%	7%

\*Differs significantly from 1954-1959 figures (Modified from Hersh<sup>84</sup>)

able, and professional experience.<sup>67</sup> We achieved better results with 6-MP in the second seven years of its use than in the first. Increased familiarity with the drug probably was an important factor.<sup>20</sup> Survival of patients with ALL treated at "centers" has been shown to be markedly superior to survival of patients treated elsewhere in the United States<sup>117</sup> and Great Britain.<sup>2</sup>

Very significant prolongation of life has been achieved in patients with ALL (Figs. 47-5A and 47-6). Before the introduction of steroids and folic acid antagonists in 1948-49,<sup>54</sup> survival was quite similar to that of AML.<sup>79,139,173</sup> It has been steadily prolonged as new drugs have been introduced; median survival now exceeds three years in some series.<sup>91,134</sup>

Whether or not a remission is achieved by therapy, there are innate features of the disease at the time of diagnosis that influence survival. Obviously, the patient who presents with life-threatening hemorrhage or infection may die before platelet transfusions or antibiotics become effective. The number of blasts in the blood also influences survival.<sup>12,19,52,65,118,188</sup> This has been recognized for more than 50 years.<sup>7</sup> Ineffectively treated patients with more than  $100 \times 10^9$  blasts/l lived an average of only one month and none survived for six months in our series. Conversely, ineffectively treated patients with fewer than  $10 \times 10^9$  blasts/l lived an average of five months and 20% lived for a year or more. Similarly, remissions lasted longer in patients with low blast counts at diagnosis than in those in whom the count was high, although the rate of remission induction in ALL was similar in the two groups.<sup>19,63</sup> Independently of the blast count, the presence of sternal tenderness was also found to be a relatively poor prognostic sign.<sup>19</sup> Since blast count could not be correlated with organ size and since organ size could not be correlated with survival in most<sup>19,125</sup> but not all<sup>77</sup> studies, it remains uncertain whether or not the total number of leukemic cells borne by the patients influences prognosis. The breakdown of undefined normal marrow release mechanisms (Chapter

2) probably is not as great in patients with few blasts in the blood as in those with many, and one might speculate that the better survival in the former group reflects better maintenance of normal control mechanisms. Age appears to be an important factor in determining response to therapy and survival.<sup>50</sup> Adults with ALL lived a slightly, although not significantly, shorter time than did children in our<sup>19</sup> and in certain other<sup>12,129</sup> series, and did significantly less well than children in other series.<sup>13,82</sup> The remission rate in patients with AML over the age of 60 may<sup>20</sup> or may not<sup>71a</sup> be lower than in younger ones, even though the general features of the disease appear to be much the same in both groups. There is some suggestion that Negro patients with ALL do less well than Caucasians.<sup>178</sup> Low platelet levels have been reported to suggest poor prognosis in some<sup>12,52</sup> but not other<sup>19,183</sup> series. Obesity and a long period of symptoms before diagnosis were regarded as indicators of poor prognosis in one study,<sup>183</sup> but duration of symptoms bore no relation to survival from the time of diagnosis in other series.<sup>19,188</sup> The presence of anergy may be a poor prognostic sign in acute leukemia,<sup>85</sup> but this has been disputed.<sup>72a</sup> There is a suggestion that females with ALL fare better than males.<sup>13</sup>

## Therapy

### General Principles

The management of acute leukemia, like that of any other fatal illness, requires all the art and scientific acumen of the physician. The physician must be skillful in management of the psychologic aspects of the disease (Chapter 55) as well as the somatic features. The goal of therapy is to reduce morbidity and prolong life. In acute leukemia this usually can be equated with induction of a complete remission(s) and maintenance of the patient in complete remission for as long as possible. During trials of remission induction, vigorous therapy of complications is mandatory. Prophylactic administration of platelets when severe thrombocytopenia is

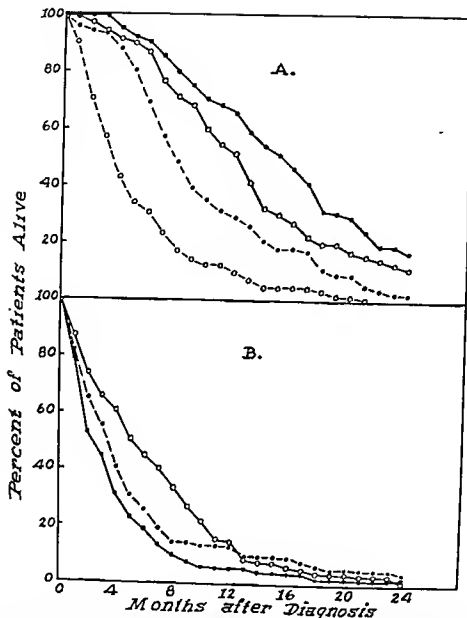


Fig 47-5 To show the steady improvement in survival of patients with acute lymphoblastic leukemia (A) in the successive periods (reading from left to right) 1947-1954 (—○—), 1954-1957 (—●—) and 1958-1964 (—○—), as well as in patients treated with a preferred method of sequential therapy (—●—) 117

The survival of patients with acute myeloblastic leukemia (B) improved only slightly in the same periods of time (1947-1954, —●—, 1954-1957, —●—, 1958-1964 —○—)

present may be advisable (Chapter 54) and serious bleeding should be treated by platelet transfusions until it has ceased (Chapter 54). Careful evaluation of fever or of any complaint suggesting infection is essential since prompt, specific antibiotic therapy is necessary in treating infection in neutropenic patients (Chapter 54). Good hydration and

urine flow as well as allopurinol administration are important in avoiding urate nephropathy (Chapter 54). Corticosteroids, vincristine, and asparaginase—useful remission-inducing agents in ALL—usually do not induce depression of normal blood cells (Chapter 55). However, all other agents in use poison normal as well as leukemic

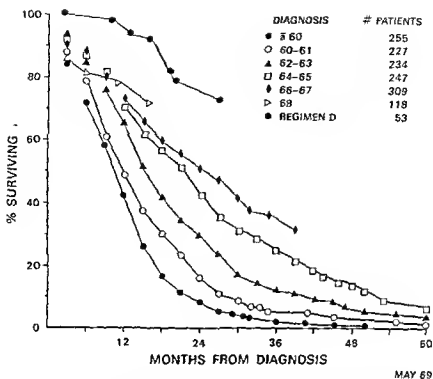


Fig 47-6. Changing survival of patients under 20 with acute lymphoblastic leukemia entered in acute leukemia group B protocols. 'Diagnosis' refers to the years when the diagnosis was made. As the years have progressed the chemotherapy has become more and more intensive and a variety of drugs have been added. Regimen D refers to children included in the 1966-67 curve who had been induced into remission with vincristine and prednisone and had received three courses of methotrexate. Prior to 1960 no child survived five years. In each subsequent grouping, the five-year survival has increased. (From Holland<sup>21</sup>; courtesy of the author and Pediatrics.)

cells. Even when remission follows their use, a period of increased thrombocytopenia and neutropenia ensues during the initial stages of therapy. It is during this cytopenic, pre-remission stage that adjunctive therapy (Chapter 54) has its greatest value. The advisability of vigorous therapy of hemorrhage and infection in patients for whom no drugs which are likely to induce remission are available is more debatable.

The original criteria agreed upon for definition of remission can be generally stated as: *Complete remission*—an asymptomatic patient with no physical abnormalities; normal hemoglobin, leukocyte, and platelet blood values; and normal-appearing bone marrow. *Partial remission*—marked improvement but with some residual evidence of disease. These were defined in some detail<sup>15</sup> so that reports from different centers would be comparable. However, these standardized criteria have not

been used routinely so that the reader must be somewhat wary of comparing the frequency of reported remissions in different series. As toxic regimens of remission-maintenance therapy have been developed (page 1491) the criteria for lack of morbidity and for normal blood values have sometimes been dropped from the definition of remission and emphasis has been placed on the absence of increased blasts in the bone marrow.

It should be noted that, even in the presence of "complete remission," careful study may reveal residual leukemic cells in organs such as the liver and kidneys.<sup>119,155</sup> In addition, it is now well recognized that meningeal infiltration can occur during remission (Chapter 54).

A decision to interrupt therapy because of thrombocytopenia or neutropenia during remission induction requires careful evaluation

of the patient as well as considerable experience with the disease. If no blasts are present in the blood or marrow, therapy should be stopped until neutropenia and thrombocytopenia improve or until blasts return. Conversely, if blasts are still present in the blood or if they are numerous in the marrow the proper course may be to continue chemotherapy at full dosage despite increasingly severe neutropenia or thrombocytopenia. With residual but seemingly reduced blasts, reduced dosage or transient interruption of therapy may be in order. While one can design hard-and-fast rules for management, as is done for experimental therapy protocols, the physician experienced in chemotherapy individualizes his decisions.

It should be emphasized that prolonging life with chemotherapy in acute leukemia need not cause a corresponding increase in the duration of morbidity during the course of the disease. If drug toxicity has been avoided during remission and if relapse is detected at an early stage, the patient may be kept in a relatively asymptomatic state from the onset of the initial remission until the terminal phase of the disease (Fig. 47-7).

### Therapy of ALL

It is generally agreed that chemotherapy should be started in all patients as soon as the diagnosis of ALL has been established. Therapy of meningeal infiltration or prophylaxis for such infiltration (Chapter 54) is as essential as systemic chemotherapy in these patients.

#### Remission Induction

Remission can be induced by a wide variety of agents, given singly or in combination (Table 47-5). Prednisone, vincristine, and asparaginase induce remission usually without toxic effects on normal marrow cells (Chapter 55). Thus, their use avoids the period of increased cytopenia which often presages remission induced by other agents. A very widely used regimen for initial treatment is prednisone-vincristine therapy; this

results in complete remission in about 90% of patients (Table 47-5). Prednisone is given by mouth in a daily dose of 1 to 2 mg/kg and need not exceed a total dose of 60 mg/day,<sup>174</sup> while vincristine is injected intravenously once a week, approximately 2 mg/m<sup>2</sup>/week, not exceeding a total dose of 2 mg<sup>50</sup> (Chapter 55). Use of these drugs is continued until complete remission has been achieved, until neurotoxicity from vincristine appears, or for a maximum of six weeks. If neurotoxicity interrupts vincristine therapy, prednisone should still be given. Complete remission usually is achieved by four weeks and, if remission is not attained by six weeks, its subsequent development is unlikely and therapy should be changed. Starting a "maintenance" drug such as 6-MP will often induce remission in patients who at that point have improved but are not in remission.<sup>19,66</sup> Larger doses of prednisone may result in slightly more rapid remission induction but do not result in a higher remission rate.<sup>113,154</sup> Intermittent prednisone therapy, though less toxic, is inferior to continuous therapy in inducing remissions.<sup>113</sup> Doses of vincristine lower than 2 mg/m<sup>2</sup>/week may induce a smaller number of remissions,<sup>60</sup> but if more than a total dose of 2 mg is given, neurotoxicity may be severe (Chapter 55). Alternatively, prednisone alone may be used. Remission is induced in more than 50% of cases (Table 47-5) and improvement occurs in almost all patients. If remission has not been achieved, vincristine can then be given; the eventual remission rate is similar to that observed when the drugs have been used in combination.<sup>153</sup>

With relapse from the first remission, prednisone and vincristine often are again effective; these drugs may induce three or more consecutive remissions (Table 47-6).<sup>107,161</sup> However, repeated use of vincristine must always be tempered by the degree of previously observed neurotoxicity. Addition of daunomycin to prednisone and vincristine in second, third, and fourth remission-induction attempts did not prove beneficial.<sup>99,175</sup>

The use of four drugs in combination, as in the "POMP" or "VAMP" regimens,<sup>32</sup>

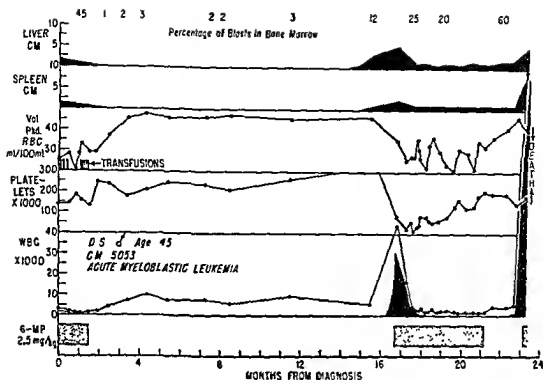


Fig. 47-7 Long remission following administration of 6-mercaptopurine in an adult patient (S D) with acute myeloblastic leukemia

results in enough toxicity that the equivocal increase in rate of remission induction does not of itself justify their use. Indeed, one of the first cooperative group studies<sup>62</sup> indicated that inducing severe toxicity with 6-MP or MTX did not enhance the remission rate. Possible justification for their use on the basis of longer duration of remission is discussed below.

When prednisone with vincristine becomes ineffective in remission induction, asparaginase probably is the drug of choice, being effective in about 50% of patients (Table 47-5). A recommended dose of asparaginase has not been established but 10,000 units/m<sup>2</sup> once a week may be as effective as higher doses and is less toxic.<sup>135</sup> The use of other agents listed in Table 47-5 for remission induction depends on whether the drug has or has not been used in maintaining prior remissions. If relapse occurs during full dosage of a chemotherapeutic agent, the drug no longer is of any future benefit to the patient except under unusual circumstances.<sup>19,65</sup> However,

MTX, when used in intermittent intravenous dosage, has induced remissions after relapse occurred during daily oral therapy.<sup>132</sup> The effectiveness of certain remission-inducing agents is heavily dependent upon the severity of the disease. Cyclophosphamide will induce remissions if it has been started early in relapse but is rarely effective in the severely ill patient<sup>133</sup> (Table 47-5).

In general, the chance of inducing a remission with a given drug is independent of whether it is used initially or in subsequent relapses. For example, the remission rate with 6-MP was virtually the same when used in untreated patients or in those relapsing from an MTX-induced remission and the same was true for MTX used initially or after a 6-MP-induced remission.<sup>1</sup>

### Maintenance Therapy

Unless effective therapy is continued during remissions induced by prednisone or prednisone and vincristine, relapse occurs

**Table 47-5. Remission Induction in Children with Acute Lymphoblastic Leukemia—First Exposure to the Drug**

Drug	Number of Patients	Remissions (% of Patients)		
		Complete	Partial	Total
Single drugs				
Hydrocortisone, <sup>148</sup> 12 mg/kg/day	54	39	22	61
Various steroids various doses <sup>19</sup>	100	—	—	71
Prednisone <sup>113</sup> 2 mg/kg/day	77	—	—	77
4 mg/kg/day	70	—	—	73
Prednisone <sup>186</sup> 2 mg/kg/day	337	—	—	69
Vincristine, <sup>158</sup> 2 mg/m <sup>2</sup>	54	30	41	71
Vincristine <sup>100</sup> 2 mg/m <sup>2</sup>	103	57	16	73
Asparaginase various doses <sup>74</sup>	137	46	5	51
Asparaginase various doses <sup>170</sup>	84	48	4	54
6-Mercaptopurine 30 mg/kg <sup>1</sup>	43	—	—	47
2.5 mg/kg <sup>94</sup>	67	45	24	69*
2.5 mg/kg <sup>74</sup>	87	47	18	65†
Methotrexate various doses <sup>1</sup>	48	22	7	29
various doses <sup>19</sup>	54	—	—	20
Cyclophosphamide 3 mg/kg/day <sup>113</sup>	73	12	19	31
patients in good condition	53	17	26	43
patients in poor condition	20	0	0	0
Daunomycin <sup>134</sup> 30/m <sup>2</sup> × 6 days	29	17	7	24
45/m <sup>2</sup> × 5 days	28	32	0	32
60/m <sup>2</sup> × 5 days	39	38	3	41
Cytosine arabinoside <sup>94</sup> various doses	51	4	22	26
Drug combinations				
Prednisone and vincristine <sup>151</sup>	63	84	5	89
Prednisone and 6-mercaptopurine <sup>188</sup>	154	82	9	91
Prednisone and cyclophosphamide <sup>56</sup>	58	59	22	81
Prednisone and daunomycin <sup>93</sup>	37	76	16	92
6 MP and methotrexate <sup>1</sup>	39	—	—	58
VAMP ‡ <sup>93</sup>	16	—	—	88
POMP ‡ <sup>93</sup>	35	—	—	94

\*Selected series, sick patients received steroids

†Remission criteria less stringent than most in the table

‡Combinations of prednisone (P), vincristine (V or O for Oncovin), 6 MP (M) and MTX (A or M for amethopterin or methotrexate) in various doses for varying times



**Table 47-6. Repeated Remission Induction in Children with Acute Lymphoblastic Leukemia Using Prednisone or Prednisone and Vincristine (All Patients Had Complete [CR] or Partial Remission [PR] during the First Trial of the Drugs)**

Repeated Trials	Remission Inducing Agents		
	Prednisone <sup>19</sup>	Prednisone <sup>163</sup>	Prednisone and Vincristine <sup>107,159</sup>
<b>Second</b>			
Number	49	48	39
% CR	—	21	67
% PR	—	19	15
Total % R	26	40	82
<b>Third</b>			
Number	13		47
% CR	—		43
% PR	—		38
Total % R	8		81
<b>Fourth</b>			
Number		18	
% CR			6
% PR			44
Total % R			50

quite quickly in ALL patients (Table 47-7). Certain drugs that are quite useful as remission-inducing agents are not effective in maintaining the remission once it has been achieved (Table 47-7). For example, in controlled clinical trials, the duration of steroid-induced remissions was not prolonged by continuing steroids after remission was achieved.<sup>97</sup> Drugs given as single agents which have proven remission-maintenance properties are MTX, 6-MP, and cyclophosphamide,<sup>56</sup> although in one study<sup>133</sup> cyclophosphamide was of questionable benefit in maintenance (Table 47-7). The timing of doses can be as important as the specific drug used. Given in maximally tolerated daily doses, MTX is inferior to 6-MP as a remission-maintenance drug,<sup>19</sup> but when MTX is given twice each week in a dosage of 30 mg/m<sup>2</sup> of body surface it becomes the best-known single agent (Table 47-7). Such observations suggest that more optimal dosage schedules might be found for other drugs. Intermittent therapy cannot be considered universally superior to daily therapy, however, since intermittent cyclophosphamide proved inferior to daily dosage in remission induction.<sup>133</sup> If one wishes to maintain remission with a minimum of toxicity the first

remission should probably be maintained with a twice weekly administration of methotrexate and the second and third with daily oral doses of 6-MP and cyclophosphamide, respectively. Drugs such as 6-MP and MTX have been given in sequential rotations every four to eight weeks in an attempt to delay development of resistance to the drugs. However, the total duration of time in remission was not increased by such regimens,<sup>63</sup> nor was it increased by combination 6-MP-MTX maintenance<sup>1,63</sup> (Table 47-7). Multiple drug and "reinduction" maintenance regimens are discussed below and experimental concepts such as immunotherapy, which has been reported to be effective in certain studies<sup>150</sup> but not in another,<sup>96</sup> are considered in Chapter 55.

### Long Remissions, "Cure," and Intensive Combination Chemotherapy

A few "cures" were reported before the advent of remission-inducing therapy in acute leukemia. Spontaneous remissions of short duration<sup>23</sup> were well documented and there was at least one convincing report of a patient remaining well for some years.<sup>146</sup>

After the introduction of chemotherapy with induction of remission, a few patients were observed who remained (and continue to remain) in remission for many years. The longest known remission was 21 years as of 1972, and at least 93 patients were known to be alive and in remission for nine or more years.<sup>27</sup> The patient with the longest remission in our series was 19 years of age when the diagnosis of ALL was made in 1959. She developed a complete remission with prednisone therapy, was maintained with daily 6-MP for approximately five years, and remains in remission. Most long-term survivors have been children with ALL, but also among them are adults with ALL and patients of all ages with AML.<sup>13,27</sup>

Such observations raise hope that a few patients with acute leukemia have been cured. The difficulty in defining a cure lies in the observation that a number of patients have been reported who experienced relapse in the fifth and sixth years of remission, and relapse after 11,<sup>55</sup> 12, and 16<sup>27</sup> years has been noted. Intensive chemotherapy (see below) probably leads to a higher percentage of patients achieving five-year remissions<sup>134</sup> than does single-drug induction and maintenance therapy.<sup>19</sup> Simply stated, the current dilemma in the treatment of patients with ALL concerns the question of whether or not patients have been cured. If so, then the morbidity and expense associated with aggressive combination chemotherapy are easily justified; if not,

**Table 47-7. Duration of Complete Remission in Children with Acute Lymphoblastic Leukemia**

<i>Method of Induction</i>	<i>Number of Patients</i>	<i>Maintenance Therapy</i>	<i>Median Duration of Remission (Weeks)</i>
Hydrocortisone <sup>143</sup>	54	None	4
Various steroids <sup>19</sup>	24	None	10
Prednisone <sup>134</sup>	99	None	8
Vincristine <sup>100</sup>	38	None	6
Vincristine <sup>100</sup>	28	Vincristine	9
Prednisone and vincristine <sup>145</sup>	20	None	8
Asparaginase <sup>170</sup>	17	None	10
Asparaginase <sup>170</sup>	17	Asparaginase	12
Prednisone <sup>19</sup>	18	6 Mercaptopurine	33
Prednisone and 6-MP <sup>46</sup>	24	6 Mercaptopurine	33
Prednisone and 6-MP <sup>43</sup>	37	6 MP and MTX	29
Prednisone and 6-MP <sup>43</sup>	34	Alternating 6 MP and MTX	26
Prednisone and cyclophosphamide <sup>54</sup>	34	Cyclophosphamide	24
Prednisone and vincristine <sup>151</sup>	27	MTX daily	14
Prednisone and vincristine <sup>28</sup>	20	MTX twice weekly (po)	45
Prednisone and vincristine <sup>28</sup>	22	MTX twice weekly (im)	38
POMP <sup>83</sup>	32	None	34
VAMP <sup>78</sup>	11	None	21
BIKE <sup>78</sup>	12	None	21
Prednisone and vincristine followed by 6-MP and MTX <sup>114</sup>	69	Alternate 6 MP and MTX	56
Prednisone and vincristine followed by 6-MP and MTX <sup>114</sup>	64	Alternate 6-MP and MTX and HN2 and actinomycin	67
Prednisone and vincristine <sup>134</sup>		6-MP and cyclophosphamide and vincristine	
	21	'full dose'	144
	21	'half dose'	71

\* POMP, VAMP, and BIKE refer to prednisone, vincristine, 6-MP, and MTX combined. Dosage is higher in POMP than in VAMP or BIKE. BIKE means two cycles of these drugs. HN2 refers to nitrogen mustard.

its justification is less apparent. The rationale of intensive combination chemotherapy is the concept that every leukemic cell can be killed, a concept presently based on certain unproven assumptions (Chapter 55). Unfortunately data adequate to make one certain of cure are not available. Cure might be defined by demonstrating a marked lessening of the chance of relapse with each successive year of remission, as has been shown in HD (Chapter 50). This has been postulated in the case in ALL<sup>27</sup> but there has been no evident reduction with time as to the chance of relapse in the series of 165 patients with ALL, known to have remained in remission for at least four years.<sup>13</sup>

Induction therapy with toxic doses of 6-MP, MTX, prednisone, and vincristine ("POMP")<sup>83</sup> has not significantly increased the percentage of remissions over that observed after prednisone and vincristine therapy (Table 47-5). However, the duration of unmaintained remission has been much longer after "POMP"-induction therapy (Table 47-7). Evidence that the duration of unmaintained remission bears a direct relation to the intensity of induction therapy is also found in the observation that "POMP"-induced remissions are longer than "VAMP" or "BIKE" remissions, regimens using the same four drugs contained in "POMP" but at lesser dosage.<sup>32, 83</sup> Intensive adjunctive therapy (Chapter 54) with frequent platelet and leukocyte transfusions, intensive antibiotic therapy for complicating infections, and prolonged hospitalization are required for the use of "POMP" and many other combinations discussed below. The high mortality associated with "VAMP" therapy in centers other than those specifically devoted to treatment of acute leukemia attests to the toxicity of the regimens.<sup>171</sup> Nevertheless, long remissions can be obtained by intensive treatment of children relapsing from remission<sup>6</sup> as well as in previously untreated patients.

Consolidation therapy employs the concept that a brief period of intensive therapy immediately following induction of complete remission should prolong the remission. For example, the effect of a brief, intensive course

of MTX following prednisone and vincristine remission induction is under study.<sup>12, 13</sup>

Reinduction therapy is a term applied to the periodic treatment of the patient who is in remission. The type of treatment is similar to that which might be given if he had experienced relapse. When at various intervals different regimens of combined drugs have been given in toxic dosage,<sup>12, 134</sup> the duration of remission has proved to be longer than that seen with single-drug maintenance. The number of available drugs (Table 47-5) and the almost limitless possibilities of time and dose schedules have led to numerous studies of different combinations of drugs for induction, consolidation, maintenance, and re-induction.<sup>12</sup> Even agents which are not effective in inducing remission in ALL, such as nitrogen mustard, BCNU, and actinomycin D, appear to prolong remissions when used in combination with known useful drugs<sup>12</sup> (Table 47-7). Addition of local irradiation to areas other than the CNS which are likely to contain "residual" leukemic cells failed to prolong remission.<sup>156</sup> The results of a few such treatment schedules are indicated in Table 47-7. Death from infection during remission is not uncommon with these regimens and seems to relate as much to immunosuppression as to neutropenia.<sup>22, 134</sup> A number of centers are giving vigorous therapy for two to three years and then discontinuing therapy in those patients who are still in remission.<sup>105, 134</sup> Fifteen children who had been in continuous, maintained remission from  $2\frac{3}{4}$  to  $3\frac{1}{2}$  years were randomized to continue or discontinue maintenance therapy.<sup>105</sup> During the following two years, relapse occurred in two of the seven who were maintained and in one of the eight who were not maintained, suggesting that long-term therapy is not necessary.

### Therapy of AML

The vast majority of patients with AML probably should be started on chemotherapy as soon as the diagnosis has been established since most are severely symptomatic or will become so shortly. Occasional patients, espe-

cially elderly ones considered by some as having "smoldering" leukemia, may remain in a relatively comfortable state with no therapy.<sup>102</sup> There is no clearcut evidence that some patients with morphologic variants of AML respond better or worse to therapy than do others.<sup>13,20</sup>

### Remission Induction

Three drugs are of unequivocal benefit in the therapy of AML—6-mercaptopurine (or its analog thioguanine [TG]), cytosine arabinoside (CA), and daunomycin (DN). These can be used as single agents, a second to be used if the first fails, or in combination.

**6-MERCAPTOPYRINE (6-MP).** This drug did not have very impressive remission-inducing properties when used as a single agent in our series of patients (Table 47-8).<sup>20</sup> Fewer than one fourth of the patients achieved a complete or partial remission. However, treatment with this drug is relatively easy and sometimes is successful for a time (Fig. 47-7). Other series of similarly treated patients indicate similar or even lower rates of remission.<sup>12,48</sup> The drug is given orally at a dose of 2.5 mg/kg per day and, unless therapy must be interrupted because of toxicity (Chapter 54), should be given for from six to eight weeks. If there has been no improvement in levels of erythrocytes, reticulocytes, platelets, or neutrophils by eight weeks, use of the drug should be stopped since there is almost no chance that remission will occur with more prolonged therapy. If any of these parameters are improving, therapy should be continued as long as improvement persists. Blasts should disappear from the blood within the first four weeks of therapy in patients who are likely to develop remission. However, modest increases in blasts in the marrow persist during partial remissions. Thioguanine, a close analog of 6-MP, has the same spectrum of action as a single agent in AML as 6-MP.<sup>104</sup> Since the metabolism of TG is not affected by allopurinol, TG is probably preferable to 6-MP during periods of allopurinol use. If remission occurs, most recommend continuing 6-MP as maintenance therapy as long as remission persists. How-

ever, there has been no study which proves that maintenance therapy prolongs remission in AML as it does in ALL (page 1491).

**CYTOSINE ARABINOSIDE (CA, ARA-C).** Given as a single agent, CA may be a slightly better remission-inducing agent than 6-MP<sup>32</sup> (Table 47-8). The presently preferred regimen appears to be one of intermittent therapy. However, a wide variety of schedules has been tried and the best method of administration is not established as yet<sup>32</sup> (Chapter 55). An example of a currently used schedule is as follows: an iv injection of 100 mg/m<sup>2</sup> total dose/day of CA is given every eight hours for a period of five days. No therapy is given for the next 10 days and this cycle is then repeated. If a remission is obtained, marked improvement is usually noted by the third cycle. As good or perhaps even better results are obtained when CA is given daily for four days in four-hour infusions of 70 to 150 mg/m<sup>2</sup>.<sup>71</sup> Whether maintenance should be with periodic courses of CA or with 6-MP, or both, is unsettled.

**DAUNOMYCIN.** Given as a single agent, daunomycin has produced a higher percentage of remission than either 6-MP or CA<sup>74</sup> (Table 47-8). When given daily for three to five days, followed by a rest and then repeated, a higher percent of remissions was induced than when it was given once a week.<sup>13</sup> The best current regimen may be to give 60 to 80 mg/m<sup>2</sup> iv once a day for three days, wait five days, and repeat the three-day courses once or twice if myeloblasts are still present. Cardiac toxicity (Chapter 55) is unusual if less than a total of 600/m<sup>2</sup> is given, but marrow toxicity (Chapter 55) is severe and somewhat unpredictable. Daunomycin alone proved superior to the four-drug combination, "POMP," in a randomized trial yielding 50% CR as compared to 29%.<sup>184</sup> Adriamycin, a close structural analog of daunomycin, has not appeared to be as useful in AML as daunomycin.<sup>33</sup>

**COMBINED CA AND 6-MP OR TG.** In the hope that the drugs would prove synergistic, or at least additive, various schedules for combined administration have been tested.

Table 47-8. Remission Induction in Acute Myeloblastic Leukemia

Drug	Number of Patients	Percent of Patients with		
		Complete Remission	Partial Remission	Total Remissions
6-Mercaptopurine <sup>20</sup>				
1952-1960	70	12	4	16
1961-1968	76	22	8	30
6-Mercaptopurine <sup>54*</sup>	31	10	6	16
Methotrexate <sup>19</sup>	34	3	3	6
6-MP and MTX <sup>42</sup>	36	14	3	17
Cytosine arabinoside <sup>32*</sup>	360	21	7	28
Daunomycin <sup>13</sup>	43	56	?	56+
Literature summation	211	34	7	34+
Daunomycin <sup>72</sup>	71	25	15	40
Cytosine arabinoside and 6-thioguanine <sup>32*</sup>	31	—	—	45
Vincristine, daunomycin and cytosine arabinoside <sup>141</sup>	23	48	4	52
L-asparaginase, daunomycin and cytosine arabinoside <sup>40</sup>	23	56	4	60
Cytosine arabinoside prednisone vincristine and cyclophosphamide (COAP) <sup>32*</sup>	45	—	—	47
Hydroxyurea, cytosine arabinoside and 6-thioguanine <sup>73†</sup>	31	42	26	68

\*Based on "evaluable" rather than total cases entered

To date, results of relatively small series suggest that their effect will be additive at best (Chapter 53) (Table 47-8), the results being those predicted on the basis of their individual effects rather than being attributable to synergism<sup>56</sup> (Table 47-8). A variety of other combinations of drugs has been tried with rather variable results (Table 47-8).<sup>13,21,32,52,70,141</sup> In 1971, results of therapy in at least 31 series of more than 20 adults with AML had been reported.<sup>70</sup> In only four such series did complete remission rate reach 50%, three with daunomycin alone and one with a four-drug combination.

**OTHER AGENTS.** The use of steroids in AML remains a controversial topic. Shortly after these agents became available it became apparent that they were primarily useful in ALL and of little benefit in AML.<sup>57</sup> Spontaneous remissions develop in AML with a frequency of approximately 5%<sup>64,151</sup> and the remission rate with steroids as a single agent did not exceed 5% in our series.<sup>19</sup> Shanbron<sup>154</sup>

likewise failed to note improvement. Other series have reported improvement with steroid therapy in a significant number of patients said to have AML (see refs 19 and 142). It has been suggested that steroids may accelerate the course of AML.<sup>55,68</sup> When combined with 6-MP it has been reported that steroids are harmful or beneficial or have no effect.<sup>19,187</sup> Considering the conflicting literature, we are unconvinced that adrenocorticosteroids have any appreciable efficacy in AML and, in view of their undesirable side effects, rarely use them. Hydroxyurea fails to induce a significant number of remissions in AML (1 in 67 trials) but, combined with prednisone, a small but significantly higher number of remissions (8 in 52 trials) occurred.<sup>142</sup>

*Methotrexate* is of little or no benefit in an oral daily dose either as a single agent<sup>19</sup> or combined with 6-MP.<sup>56</sup> However, when given orally or iv, at fairly toxic dosage on an intermittent basis, remissions have been reported.<sup>177</sup> *Methyl-GAG* (methylglyoxal-

bis-guanyldiazotization)<sup>115</sup> induces a significant number of remissions in AML but is exceedingly toxic (Chapter 55). Cyclophosphamide,<sup>83</sup> vincristine,<sup>83</sup> and asparaginase<sup>83,128,169</sup> are among the rather large group of agents (Chapter 55) that have been administered in AML with modest or equivocal effects.

The duration of remissions, even complete remission, may be rather short. In one review of 96 complete remissions in AML, median duration of remission was 4.2 months with a mean of 6.3 months and a range of 1 to 18 months.<sup>153</sup>

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## Chronic Myelocytic Leukemia

### Classification

#### ✓ Presenting Features

##### Symptoms

##### Physical Findings

##### Laboratory Studies

#### Differential Diagnosis ✓

#### Course

##### Duration of Survival

##### Causes of Death

##### Factors Influencing Prognosis

#### Therapy

##### Bussulfan

##### Irradiation

##### Other Forms of Therapy

#### Atypical CML

##### CML in Infants

#### Morphologic Variants of CML

#### Blast Crisis

are young or middle-aged adults. This form of leukemia is more frequent in males than in females by a ratio of approximately 3:2.

### Classification

In terms of predictability of the course and responsiveness to therapy, at least two types of CML can be distinguished on the basis of the presence or absence of the Philadelphia chromosome (Table 48-1). There also are differences in clinical manifestations, course, and survival in patients whether they do or do not have the  $Ph^1$  abnormality (page 1512). A possible sub-set of the  $Ph^1$ -positive group has been suggested.<sup>36</sup> These patients lack the Y chromosome. They may be designated as 45, XO,  $Ph^1$ , indicating that they are aneuploid, due to a missing Y chromosome (XO), and have the  $Ph^1$  abnormality.<sup>36</sup> There is a suggestion that 45, XO,  $Ph^1$  confers a particularly good prognosis even though the hematologic and clinical findings otherwise seem to be identical to those of 46, XY,  $Ph^1$  patients (page 1509).<sup>36</sup> In addition, eosinophilia, basophilia, or monocytosis has been so prominent in some patients that such subtypes have been distinguished (page 1513).

### Presenting Features

The symptoms, signs, and laboratory findings present at the time of diagnosis in patients observed at the University of Utah

THE historical development, definition, and classification of leukemia in general as well as the specific place of chronic myelocytic leukemia (CML) in such a classification have been discussed, as have such aspects of CML as its possible clonal nature, etiology, pathogenesis, and incidence (Chapter 46). Chronic myelocytic leukemia is characterized by extreme elevation of the leukocyte count as a result of the presence of increased numbers of all forms of mature and immature granulocytes. It can be observed at any age, but increases steadily in age-adjusted frequency throughout life. Thus, considering the age distribution of the population of the United States, most patients seen with CML

**Table 48-1. A Classification of Chronic Myelocytic Leukemia (CML)**

<i>Clinical variants</i>
Typical CML (Philadelphia chromosome present)
Atypical CML (Philadelphia chromosome absent)
CML in infants
<i>Morphologic variants</i>
Chronic eosinophilic leukemia
Chronic basophilic leukemia
Chronic monocytic leukemia
Chronic neutrophilic leukemia

(Tables 48-2 and 3)<sup>75</sup> are quite similar to those reported from other institutions.<sup>6,26,34,50,88,103,121,124</sup>

### Symptoms

The diagnosis of CML may be made at a time when the patient is asymptomatic. As in a British series,<sup>124</sup> in 15% of our series<sup>75</sup> the diagnosis was made at such a time. These patients are found to have an elevated leukocyte count or a palpable spleen on routine examination or in conjunction with examination for unrelated illnesses. Symptoms may develop rapidly, fatigue and anemia appearing within six months after the diagnosis has been made. However, an asymptomatic period of some years' duration may be observed occasionally.<sup>12,77,81,123</sup> In patients in whom the diagnosis is made at an early stage the great majority of the blood cells are mature; only rarely are metamyelocytes and myelocytes seen.

The median duration of symptoms before the diagnosis was established was three months in our series of patients.<sup>75</sup> This is a much shorter pre-diagnostic, symptomatic period than had been noted in earlier series.<sup>79,105</sup> Perhaps, because of improving medical care patterns, the diagnosis is made at an earlier stage of disease at the present time than it was some years ago. Typical presenting symptoms of patients with CML are fatigue and/or discovery of a mass in the left upper quadrant of the abdomen, or discomfort in the left upper quadrant. Less

**Table 48-2. Symptoms and Signs Observed at Time of Diagnosis in 81 Patients with CML\***

	% of Patients
<i>Symptoms at diagnosis</i>	
Fatigue	83
Weight loss	61
Abdominal fullness	38
Easy bruising or bleeding	35
Abdominal pain	33
<i>Physical findings</i>	
Splenomegaly	95
Hepatomegaly	48
Sternal tenderness	78
Purpura	27
Retinal hemorrhage	21
Fever	11
Palpable lymph nodes	64
Palpable lymph nodes exceeding 1 cm in diameter	8

\*Patients seen in the University of Utah Hematology Clinic<sup>75</sup>

commonly, symptoms related to peptic ulceration, bleeding, thrombosis, bone pain, arthralgia, or leukemic infiltration in sites other than the spleen, such as the skin, are the primary complaints. As in polycythemia vera

**Table 48-3. Blood Cell Values at Time of Diagnosis of CML (81 Patients)\***

<i>Leukocytes</i>	
Total count, median	$161 \times 10^3/l$
range	$27-1,076 \times 10^3/l$
% Myeloblasts, median	2%
range	0-12%
% Promyelocytes, median	4%
range	0-20%
<i>Red cells</i>	
% of patients anemic	84%
VPRC 0.30 l/l or less	41%
VPRC 0.20 l/l or less	5%
Nucleated RBC seen	64%
Reticulocytes > 3%	52%
<i>Platelets</i>	
Less than $150 \times 10^3/l$	13%
$150-450 \times 10^3/l$	30%
$> 450 \times 10^3/l$	57%

\*Patients seen at the University of Utah Hematology Clinic<sup>75</sup>

(Chapter 30), compared with the general population there is a higher frequency of peptic ulceration of the stomach or duodenum in patients with CML, but the reason for this association is unknown. Fourteen percent of our patients with CML had radiologically documented peptic ulcers and others had symptoms suggestive of peptic ulceration. Complaints of bone pain or symptoms related to compression of the spinal cord<sup>40</sup> or priapism<sup>26,40</sup> are unusual, but are quite distressing when present. Anorexia and weight loss are common, but rarely are severe.<sup>34</sup> Fever may occur (Table 48-2), but it is rarely due to a documented infection until late in the course of the disease.<sup>9</sup>

### Physical Findings

The most common signs are splenomegaly, pallor, and sternal tenderness (Table 48-2). Hepatomegaly, lymphadenopathy, purpura, and fundic hemorrhages also are detected frequently. Roentgenograms of the lungs or of bone usually show no abnormality. The spleen is quite variable in size ranging from a just palpable tip to a mass filling the left side of the abdomen. In 71% of patients in one series<sup>21</sup> and in 40% of our patients<sup>75</sup> the spleen extended more than 10 cm below the costal margin at the time of diagnosis. Spleen size correlates reasonably well in a positive fashion with the height of the leukocyte count. The spleen is quite firm, usually non-tender unless splenic infarction is present (Chapter 54), and the splenic hilum (notch) may be palpable. Sternal tenderness is a reliable sign of disease and usually is limited to a small area, most commonly in the mid-body of the sternum. If true sternal tenderness is present the patient will withdraw or spontaneously complain when firm pressure is applied to the tender area, but he will have been previously unaware of its existence. Palpable lymph nodes are present in the majority of patients, but rarely are large. Nodes estimated to be greater than 1 cm in largest diameter were present in less than 10% of our patients.<sup>75</sup>

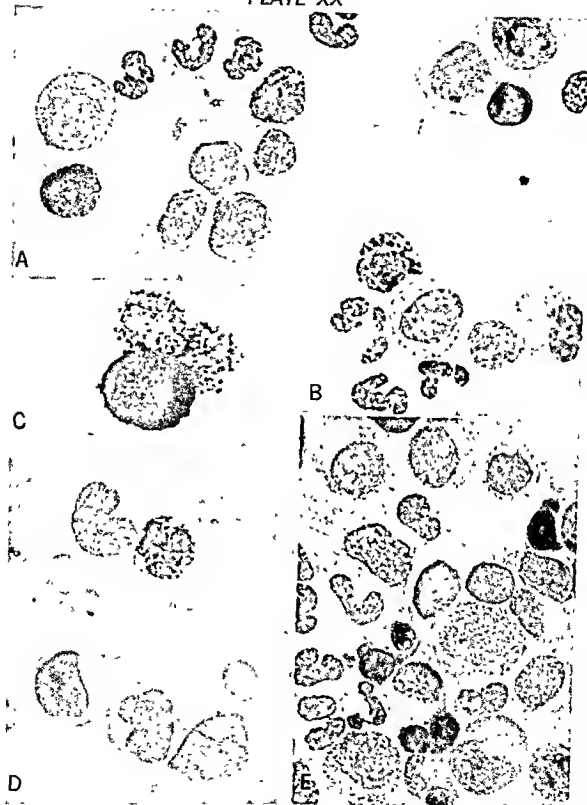
### Laboratory Studies

The leukocyte count (Table 48-3) is higher than  $100 \times 10^9/l$  in 62%<sup>75</sup> to 90%<sup>124</sup> of patients at the time the diagnosis is made and may be in excess of  $1000 \times 10^9/l$ . All stages of the neutrophilic series from myeloblasts to segmented neutrophils usually are present. Segmented and band neutrophils usually are the most common cells, followed in decreasing order by metamyelocytes, myelocytes, promyelocytes, and myeloblasts. Promyelocytes and myeloblasts make up only a small percentage of the total number of cells until blast crisis supervenes. However, there is a direct relationship between the height of the leukocyte count and the percentage of immature cells at the time of diagnosis.<sup>72,75</sup> In excess of 20% blasts and promyelocytes often is considered to signify blast crisis (page 1514), but that percentage may be present in previously untreated patients with a very high leukocyte count in the absence of blast crisis. Rarely, mature neutrophils remain the predominant blood leukocyte throughout the course of the disease (chronic neutrophilic leukemia<sup>19,27,81,107</sup>). Basophils are increased in absolute number in almost all patients as are eosinophils and monocytes and the latter cells may be mistaken for myelocytes. The absolute lymphocyte count usually is within normal limits.

In most untreated patients the leukocyte count increases progressively (Fig. 48-1). However, in some, cyclic variations in the count are noted<sup>215,80,118</sup> (Fig. 48-2). The count may rise and then fall by more than  $200 \times 10^9/l$  during such cycles and cyclical changes have been noted during therapy<sup>64</sup> as well as in untreated patients.

Anemia is present at the time of diagnosis in most patients, but is often mild and transfusion was required initially in fewer than 10%<sup>71,124</sup> (Table 48-3). The anemia is normochromic, red cell shape appears normal on blood smears, and the absolute reticulocyte count is normal or slightly elevated (Table 48-3). Only very rarely<sup>73</sup> is a hemolytic anemia present. Nucleated red cells may be

# PLATE XX



Blood from patients with leukemia (A, B, C, D) contrasted with normal bone marrow (E). A and B are from patients with chronic myelocytic leukemia (Wright's stain,  $\times 1000$ ). C and D are blood smears from patients with acute myeloblastic (C) and acute monocytic (D) leukemia and show a positive peroxidase reaction in all three cells from the patient with myeloblastic leukemia and in a single cell from the one with monocytic leukemia. E shows normal bone marrow elements (Wright's stain,  $\times 1000$ ).

most patients. Differential counts from marrow smears reveal an increased myeloid to erythroid ratio, but fairly orderly neutrophil maturation is found in most patients. The proportion of immature to mature neutrophils tends to be higher in marrow than in blood (Table 48-4),<sup>8,123</sup> suggesting that the barrier to release of immature cells (Chapter 2) is not lost completely. Detailed morphologic study, as by electron microscopy, shows certain abnormalities of the neutrophilic series. There is evidence that cytoplasmic maturation is more rapid than nuclear maturation, there being a greater than normal number of granules in promyelocytes in contrast to their immature-appearing nuclei.<sup>2</sup> Blasts and promyelocytes from patients with CML are more motile and have greater phagocytic potential than those from normal subjects, features which probably reflect cytoplasmic maturation.<sup>92</sup> Rod-shaped granules are more frequent than normal.<sup>94</sup> Auer rods are observed occasionally,<sup>12,121</sup> but by no means as frequently as in AML (Chapter 47). Barr bodies are less common in mature neutrophils in females with CML than in normal females, but are said to increase in frequency following therapy.<sup>116</sup> Pelger-Huet-like anomalies

(Chapter 42) may be observed, primarily in later stages of the disease.<sup>23,25,51</sup> A patient with CML and cells containing the Alder-Reilly leukocyte anomaly (Chapter 42) has been reported, but this patient was thought to have had the anomaly prior to developing CML.<sup>74</sup>

*The Philadelphia chromosome* (Chapter 46) has been reported to be present in 70 to 90%<sup>4</sup> of patients with CML.<sup>28,102,120</sup> When present, two thirds of the patients have the defect in all metaphases seen in direct preparations of marrow, while in one third a small percentage of normal metaphases is also seen.<sup>120</sup>

Erythrocyte development is morphologically normal, as is megakaryocyte maturation. Large abnormal-appearing histiocytic cells similar to those found in Gaucher's disease may be observed.<sup>62,71,98</sup> Their origin may be similar to that of Gaucher cells in that increased turnover of neutrophils produces excess cerebroside, which must be stored.<sup>62,98</sup> However, when examined with the electron microscope the cytoplasmic inclusions have a different configuration than those of Gaucher cells, so the stored substance may be different from the cerebroside of Gaucher's disease.<sup>71</sup> In one patient with

**Table 48-4. Comparison of Percentages of Various Stages of Neutrophilic Granulocytes in Marrow and Blood of 11 Patients with Chronic Myelocytic Leukemia\***

	Myeloblasts Promyelocytes Myelocytes (%)	Metamyelocytes (%)	Band and Segmented Neutrophils (%)
Mean of 11			
Blood	27	23	50
Marrow	38	33	29
Patient with largest difference			
Blood	2	2	94
Marrow	33	38	29
Patient with least difference			
Blood	26	26	48
Marrow	29	29	42

\*Blood and marrow samples obtained on same day in untreated patients (Adapted from Boggs<sup>8</sup>)

CML, lacto-, rather than glucocerebroside, predominated.<sup>24a</sup>

Increased reticulum fibers may be present, but frank myelofibrosis is unusual at the time of diagnosis although it develops with some frequency later in the course of the disease (page 1506).<sup>43</sup>

*Leukocyte alkaline phosphatase (LAP)* is abnormally low and may actually be absent<sup>24</sup> in approximately 90% of patients with CML. Low LAP and Ph<sup>1</sup> positivity do not necessarily correlate; i.e., LAP may be low in a Ph<sup>1</sup>(-) patient<sup>102</sup> and may be normal or high in a Ph<sup>1</sup>(+) patient.<sup>43</sup> There is some evidence that the enzyme is chemically different in CML cells than in normal cells.<sup>11</sup> The LAP may increase in response to the development of unrelated diseases, such as ulcerative colitis, in patients with CML.<sup>97</sup> When cells from patients with CML are cultured in diffusion chambers, LAP increases, thus suggesting that there is no intrinsic defect in the ability of the cell to synthesize the enzyme.<sup>21a</sup>

*Uric acid* often is moderately elevated in the serum and almost invariably increased in the urine; gout may occur (Chapter 54). However, urate nephropathy with oliguria or anuria is unusual even during therapy so that allopurinol is not needed as often as in acute leukemia. Artifacts *hyperkalemia* may be present, probably due to in vitro potassium release from platelets as blood is allowed to stand, and artifacts *hypoglycemia* may be due to leukocyte glucose utilization in vitro.<sup>29</sup>

*Histamine* and histamine metabolite levels are elevated in plasma and leukocytes in most patients with CML<sup>4</sup>; this has been considered a possible explanation for such findings as peptic ulceration, pruritus, and asthma.<sup>41,106</sup> However, in a group of patients with CML with no symptoms attributable to histamine, plasma histamine levels averaged more than three times normal, a finding which raises doubts that any symptoms can be attributed to the raised levels of histamine in CML.<sup>114</sup> The level of histamine in blood tends to reflect the numbers of basophils.<sup>4,114</sup>

*Serum albumin* usually is normal and gamma globulin often is moderately elevated, but paraprotein spikes rarely are encoun-

tered.<sup>96</sup> *Hypercalcemia*, usually associated with bone lesions, has been described occasionally.<sup>3,71a</sup> (Chapter 54).

*Serum vitamin B<sub>12</sub>* levels as well as both total and unsaturated B<sub>12</sub>-binding capacity of serum are increased in CML, primarily reflecting elevated levels of transcobalamin I or of a third serum vitamin B<sub>12</sub>-binding protein.<sup>20,47</sup> (Chapter 4, page 139). These proteins may be synthesized by mature or maturing neutrophils<sup>109,113</sup> and their degree of increase may reflect the size of the total leukocyte pool.<sup>21</sup> Whether they are chemically different from those found in normal blood is uncertain.<sup>78,108</sup>

Transcobalamin II, the vitamin B<sub>12</sub> transport protein, is not increased in CML<sup>93</sup> and may be decreased.<sup>45</sup>

## Differential Diagnosis

A variety of conditions are associated with a blood leukocyte pattern that may mimic the pattern seen in CML. Most "leukemoid" reactions suggest CML rather than other varieties of leukemia. As discussed in Chapter 41, a variety of infections and carcinomas as well as other conditions may produce extreme degrees of neutrophilia. In most instances, absence of sternal tenderness, splenomegaly, basophilia, eosinophilia, and thrombocytosis suggests the presence of a leukemoid reaction rather than CML. A smaller proportion of the blood leukocytes is immature in most patients with leukemoid reactions, as compared to the usual patient with CML, but this is not always the case.<sup>46</sup> Cytogenetic studies of the marrow cells and LAP levels are the most definitive means of distinguishing CML from a leukemoid reaction. If the LAP is normal or raised as it is in most leukemoid reactions and if the Ph<sup>1</sup> chromosome is absent, CML is most unlikely. However, decreased LAP was reported in a patient with miliary tuberculosis.<sup>107</sup> Serum vitamin B<sub>12</sub> and transcobalamin levels may be increased in leukemoid reactions as they are in CML.<sup>18,49</sup>

In polycythemia vera (PV, Chapter 30) and in idiopathic myelofibrosis (IMF, Chapter

57), leukocytosis comparable to that seen in CML may be present, an observation which led to the concept that these are interrelated conditions constituting a "myeloproliferative disorder" (Chapter 46). Symptoms and signs of the three diseases may be quite similar although splenomegaly tends to be more extreme in IMF than in CML or PV. The size of the spleen tends to be directly proportional to the magnitude of the leukocyte count in CML so that the presence of a very large spleen with a leukocyte count of less than  $100 \times 10^9/l$  is suggestive of IMF rather than CML. The degree of immaturity of the neutrophil series, basophilia, eosinophilia, and thrombocytosis may be equal in all three conditions. However, a significant degree of anisocytosis and poikilocytosis and the presence of large numbers of nucleated red blood cells suggest IMF. A substantial amount of fibrosis in marrow biopsy specimens also suggests IMF. While fibrosis may develop during the course of CML or PV it rarely is a prominent finding at the time of diagnosis.<sup>41</sup> An abnormally low LAP makes PV an unlikely diagnosis, but the LAP may sometimes be low in IMF as it usually is in CML. The presence of the Ph<sup>1</sup> chromosome strongly favors CML although it has been described in occasional patients thought to have PV or IMF (Chapters 46 and 57). As noted below (page 1512), in patients thought to have CML, but without the Ph<sup>1</sup> chromosome, the course may be similar to that of IMF, at least as judged by their poor response to busulfan.

## Course

Until blastic crisis supervenes the symptoms and most physical and laboratory abnormalities of CML can be controlled by therapy. However, there is little evidence to suggest that therapy appreciably increases the survival time.

### Duration of Survival

Minot et al<sup>79</sup> reported the first survival figures for a large series of patients with

CML. Only those who died were included in their study. The 78 patients who were treated with irradiation, usually radium, lived an average of 3.5 years from onset of symptoms as compared to 3.0 years for those not irradiated. Minot and associates' conclusion that "irradiation has had little effect on prolonging survival" is equally applicable to other forms of therapy used in later years. Shimkin et al<sup>105</sup> found no change in survival in CML patients treated between 1910 and 1948 and in a review of the literature noted that mean survival from symptomatic onset to death ranged from 3.0 to 3.9 years. Survival figures reported more recently do not differ significantly from those reported 50 years ago.<sup>21b,40,61,75,79a,124</sup> However, in a controlled study, patients on busulfan therapy were found to live longer than those treated with splenic irradiation (Fig. 48-3).<sup>124</sup> It is conceivable that irradiation in contrast to busulfan may hasten the onset of blast crisis, thereby limiting survival.<sup>21b</sup>

Median survival of our patients to 1964 is shown in Figure 48-4. A more up-to-date analysis of 121 cases (to 1970) showed 37 months' 50% survival from time of diagnosis for all patients and 42 months for those treated with busulfan only.<sup>72a</sup> Noteworthy is the much longer survival of some patients, as illustrated in Figure 48-5.

### Causes of Death

The usual cause of death is infection or hemorrhage, often in association with blast crisis. In most instances these complications relate to the development of neutropenia or thrombocytopenia, which appears as blast crisis develops (page 1513). Other patients develop neutropenia and thrombocytopenia secondary to progressive fibrosis of the marrow.<sup>43</sup> In the terminal stages of CML, such patients may be clinically indistinguishable from those presenting with idiopathic myelofibrosis (Chapter 57). Patients in blast crisis may die as the result of formation of leukostatic lesions in the vessels of various organs,<sup>13a</sup> similar to those seen in AML (Chapter 47). A few patients die as a consequence



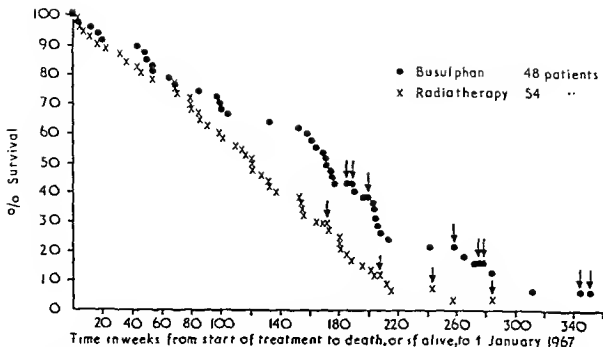


Fig 48-3. Survival of a series of patients with CML randomly selected to receive radiotherapy to the spleen (54 patients) or busulfan (48 patients). Arrows denote patients still alive at the time report was issued. (From Galton,<sup>32</sup> courtesy of the author and Grune & Stratton, Inc.)

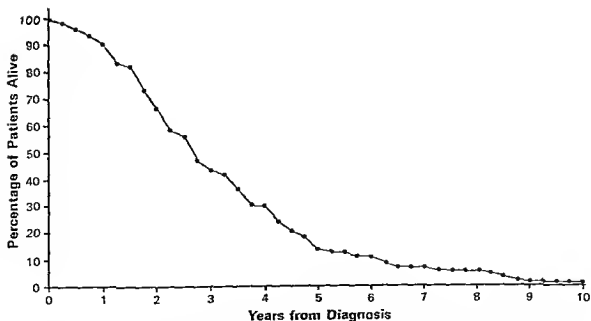


Fig 48-4. Actuarial survival of 106 patients with CML treated at the University of Utah (1944-1964). Seventy-eight patients were known to be dead, 15 were living, and 13 were lost to follow-up. Median survival was 32 months, 15% lived for five years and only two patients are known to have lived more than 10 years.

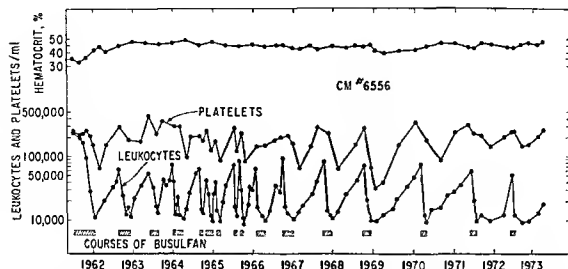


Fig 48-5 Effects of intermittent therapy with busulfan on VPRC WBC, and platelet count during a 13 year course. The patient, 80 years of age, is still alive in 1975 and remains asymptomatic between courses of busulfan. Note that he has received no medication for approximately two thirds of his prolonged illness. Ph<sup>1</sup> chromosome is positive.

of marrow aplasia induced by therapy. If intermittent, rather than maintenance, therapy is employed, and if the patient is adequately observed at frequent intervals during therapy this cause of death should be quite rare. A few patients die of causes apparently unrelated to leukemia,<sup>79a</sup> but this is less common than in CLL since the course of CML is shorter and the average age of patients is lower than in CLL (Chapter 49).

#### Factors Influencing Prognosis

There are no wholly reliable means of predicting the duration of life or the time of onset of blast crisis from features present at the time of diagnosis, although platelet count<sup>75</sup> and chromosome studies (see page 1512) are helpful. In our series,<sup>75</sup> for nine patients who were initially thrombocytopenic, median survival from the time of diagnosis was less than 12 months (range 6 to 61 months); in 21 patients with a normal platelet count, median survival was 30 months (range 8 to 81); and in 39 patients with thrombocytosis, median survival exceeded 36 months (range 14 to 142). This suggests that if thrombocytosis has any prognostic implication it is a favorable rather than an unfavor-

able<sup>5</sup> sign. We could not confirm the suggestion that hepatomegaly,<sup>79a</sup> severe anemia,<sup>86</sup> or normoblasts in the blood<sup>113</sup> are poor prognostic signs and could find no significant relationship between age, duration of symptoms, degree of leukocytosis, splenomegaly, or percentage of blasts in the blood to the duration of survival from the time the diagnosis was made until death occurred. There was a suggestion that absence of basophils and/or eosinophils from blood smears was a poor prognostic sign. This may relate to the influence of the presence or absence of the Ph<sup>1</sup> chromosome defect (page 1512). A rising percentage of blood basophils<sup>28</sup> and sparse basophilic granulation<sup>39</sup> have been suggested as developments that may herald the onset of blast crisis. The rapidity of the rise in leukocyte count after completion of the first course of radiotherapy or busulfan usually has been inversely correlated with duration of survival,<sup>63,83</sup> but not always (Fig. 48-5). It may be significant that four of eight patients who were found to have increased reticulum fibers on marrow biopsy when the diagnosis was made died in blast crisis within one year.<sup>43</sup> A multifactorial scoring method for gauging the severity of disease has been suggested.<sup>89</sup> However, when applied to our

series of patients this had no better predictive value than the platelet count alone.<sup>75</sup>

For the present, the presence or absence of the Ph<sup>1</sup> chromosome appears to be the best prognostic indicator (see atypical CML, page 1512). As mentioned earlier, the absence of the Y chromosome in addition to the Ph<sup>1</sup> abnormality, 45, XO, Ph<sup>1</sup> (page 1500) may indicate a good prognosis. A summary of survival in nine such reported patients for whom survival data were available indicated that one died at 18 months and two were living less than three years from the time of diagnosis, but six of the nine lived or were living more than four years, four of these more than seven years and two more than ten years after diagnosis.<sup>36</sup>

## Therapy

BUSULFAN (MYLERAN).<sup>32, 33</sup> This appears to be the therapy of choice on the basis of cost, ease of administration, and relative freedom from toxic effects. In controlled comparative trials of busulfan in CML patients, this drug was probably superior to chlorambucil<sup>44, 101</sup> and significantly superior to cyclophosphamide<sup>63</sup> or irradiation of the spleen (Fig. 48-3).<sup>121</sup>

Busulfan is given in a single, daily oral dose of 4 mg to adults. In the event that children with CML or patients with thrombocytosis due to myelofibrosis (Chapter 57) are to be treated with busulfan, the dosage should be reduced. In the usual patient with CML the leukocyte count begins to decrease in the second or third week of therapy. If the count has not begun to decrease by the fourth week, increasing the dose to 6 mg/day may be advisable. Once the count begins to fall it decreases exponentially although the steepness of the slope varies from one patient to another (Fig. 48-6). At a dose of 4 mg/day, use of the drug can be continued with relative safety until the total leukocyte count reaches  $8.0$  to  $10.0 \times 10^9/l$ . Since the decline may continue after busulfan administration is stopped, therapy should always be interrupted when the count reaches this level to avoid inducing neutropenia. It is only rarely

that an increase in dose of busulfan above 4 mg/day may be necessary; doses greater than this are given either because of a failure of the leukocyte count to decrease by the fourth week or because the count declines but stabilizes at levels higher than  $20.0 \times 10^9/l$ . This, however, occurs very rarely. Development of apparent resistance to busulfan is quite rare (page 1511).

By the time normal or nearly normal leukocyte counts have been reached, immature neutrophils largely have disappeared from the blood and the differential count has returned to normal in most CML patients. However, in some, immature cells persist in the blood. Persistence of basophilia is common. The platelet count, if higher than normal, declines parallel with the leukocyte count (Fig. 48-5). If it falls below normal limits, therapy should be interrupted and resumed at a lower dose after the count has returned to normal. Anemia usually lessens as the leukocyte count decreases and has often disappeared by the time therapy is stopped (Fig. 48-6). The spleen almost invariably decreases in size, but a palpable spleen persists in some patients. Sternal tenderness usually disappears.

Thus, by the time the leukocyte count nears or becomes normal, most patients are asymptomatic and few signs remain. Most however, cannot be considered to be in complete remission in that a diagnosis of CML can still be made. In addition to some residual splenomegaly, basophilia, or persistent immature cells in the blood, the Ph<sup>1</sup> chromosome defect is still demonstrable in marrow and LAP usually remains low during remission.

Once drug therapy has been stopped the patient is observed at intervals of several months for a rising leukocyte count or for return of anemia or symptoms. Since patients usually become symptomatic shortly after the leukocyte count has risen to  $50.0 \times 10^9/l$  it is our practice to reinstitute therapy when that level has been reached. The duration of remission while treatment is withheld is quite variable, but averages more than six months when the leukocyte count has been reduced to less than  $10.0 \times 10^9/l$ .<sup>53</sup> Longer remis-

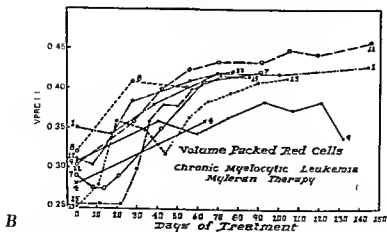
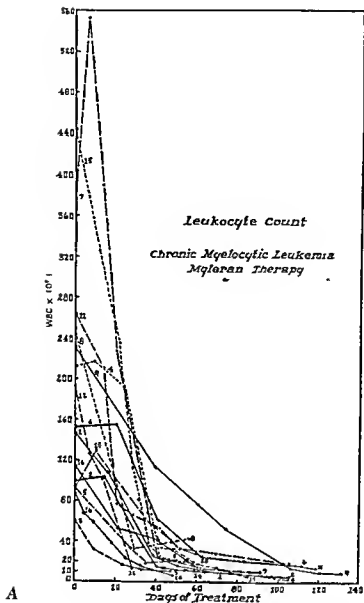


Fig 48.6 Changes in the blood in a series of patients with chronic myelocytic leukemia treated with busulfan. A, The rapid drop in the number of leukocytes. B, the simultaneous decrease of anemia as manifested by the rise in volume of packed red cells. In most patients the anemia was relieved completely.

sions have been observed in patients in whom low-normal or neutropenic levels were inadvertently induced. Indeed, remission persisting for as long as nine years has been reported after hypoplasia was induced with busulfan therapy followed by splenic irradiation.<sup>76</sup> Busulfan-induced hypoplasia may be quite prolonged and the possible hazards of severe neutropenia and thrombocytopenia are such that these low levels are best avoided.

The number of courses of busulfan that can be given in this intermittent manner appears limitless for all practical purposes. In Figure 48-5 the course of one of our longest surviving patients with CML is portrayed. To date he has received 17 courses during 13 years. There is a tendency for the duration of remission to decline with repeated courses of therapy,<sup>53</sup> but this is not always the case (Fig. 48-5). The uncommon, but severe idiosyncratic toxic reactions to busulfan such as pulmonary fibrosis are even more uncommon in patients treated on an intermittent rather than on a continuous basis, but hyperpigmentation of the skin is usual after multiple courses of an intermittent regimen.

Busulfan also has been used as maintenance therapy after the first remission has been induced.<sup>124</sup> When this is done, as the leukocyte count nears normal the dosage is reduced, but use of the drug is continued. Dosage is varied until a dose is found that keeps the leukocyte count approximately normal and therapy is continued indefinitely. The disadvantages of maintenance therapy as compared to intermittent therapy appear to outweigh any theoretical advantages. Patients on maintenance therapy<sup>124</sup> must be seen and have their blood examined at frequent, regular intervals for adjustment of dose as well as for detection of developing cytopenia at an early stage. Busulfan is somewhat unpredictable in producing pancytopenia and severe aplasia may develop after prolonged periods of seemingly satisfactory maintenance dosage. Most of the serious toxic effects of busulfan therapy have been reported in patients receiving maintenance therapy (Chapter 55).

Occasional patients prove resistant to busulfan or become resistant after repeated

courses in the absence of blast crisis.<sup>124</sup> In such patients other modes of therapy may prove effective (see below).

**IRRADIATION.** The spleen may be irradiated,<sup>53,124</sup> the whole body may be irradiated,<sup>1</sup> radioactive phosphorus (<sup>32</sup>P)<sup>50</sup> may be given, or extracorporeal irradiation of the blood (Chapter 55) may be employed. Splenic irradiation, usually approximately 1000 rads, not only reduces the size of the spleen, but also reduces the leukocyte count. It is thought that this is not a true abscopal effect (see Chapter 55), but, rather, is due to irradiation of immature neutrophils as they migrate through the spleen (Chapter 46). Thus, in a sense splenic irradiation and extracorporeal irradiation of the blood may be acting in a similar manner. Radioactive phosphorus localizes in marrow cells and may induce remission in CML.<sup>50,84</sup> Radiation of the spleen following chemotherapy may lead to severe marrow hypoplasia in some patients.<sup>122</sup>

**OTHER FORMS OF THERAPY.** Other forms of therapy also are effective, although none has greater proven effectiveness than busulfan.<sup>113a</sup> Chlorambucil, given in an oral daily dose of 12 mg, produces effects quite similar to those of busulfan.<sup>44,124</sup> Six-mercaptopurine<sup>57</sup> is a fairly effective agent when given as an oral dose of 2.5 mg/kg day. Actually it seems likely that any of the alkylating agents or antimetabolites will influence CML since various chemotherapeutic agents such as nitrogen mustard,<sup>34</sup> uracil mustard,<sup>31</sup> cyclophosphamide,<sup>31,63</sup> melphalan,<sup>104</sup> dibromomannitol,<sup>10</sup> hydroxyurea,<sup>65</sup> and mitomycin C<sup>56</sup> all have been shown to have some effect on the disease, as have such nonspecific cell poisons as benzol and arsenic.<sup>50,124</sup> Simply removing large numbers of blood leukocytes by leukopheresis may induce improvement.<sup>14</sup>

Resistance to busulfan is quite unusual, as noted above, but when it does occur, response to another agent such as melphalan<sup>104</sup> may still be obtained. Melphalan is administered in much the same manner as busulfan in a dose of 2 mg/day; use of the drug should be discontinued when the leukocyte count has decreased to  $8.0$  to  $10.0 \times 10^9/l$ . Hydroxy-

urea has proved to be useful,<sup>65</sup> but has the disadvantage that therapy must be continued indefinitely since the leukocyte count begins to rise almost as soon as its administration is discontinued.

## Atypical CML

There is increasing evidence that in patients who present a CML-like picture, but in whom the  $\text{Ph}^1$  chromosome is absent, prognosis is less good and the course is atypical as compared with that of patients in whom the chromosome defect is present.<sup>28,66,120</sup> Patients with  $\text{Ph}^1 (+)$  cells tend to differ from those without this defect in having higher leukocyte counts, a smaller percent of myeloblasts in the blood and marrow, greater degrees of thrombocytosis, basophilia and marrow cellularity, and lower LAP scores.<sup>28</sup> Muramidase excretion in urine tends to be increased in  $\text{Ph}^1 (-)$  patients, but not in those with  $\text{Ph}^1 (+)$ .<sup>91</sup>

Thus the presence or absence of the  $\text{Ph}^1$  chromosome appears to be of prognostic importance. Of 76 patients with a provisional diagnosis of CML referred to Roswell Park Memorial Institute between 1959 and 1967, 15 were found to have idiopathic myelofibrosis, AML, or striking degrees of eosinophilia.<sup>24</sup> The remaining 61 were considered to have CML by the usual clinical criteria; 43 were  $\text{Ph}^1 (+)$  and 18 were  $\text{Ph}^1 (-)$ . The median survival of the  $\text{Ph}^1 (+)$  patients was 40 months as compared to 8 months in the  $\text{Ph}^1 (-)$  group (Fig. 48-7). In another study, median survival was 45 months for  $\text{Ph}^1 (+)$  patients and 18 months for  $\text{Ph}^1 (-)$  patients.<sup>120</sup>

Certain patients with what appears to be best described as idiopathic myelofibrosis (Chapter 57) or with antecedent polycythemia vera (Chapter 30) develop a blood picture similar to that seen in CML subjects (page 1505). Separation of this group from atypical,  $\text{Ph}^1 (-)$  CML may be difficult.

If additional experience continues to give evidence of differences between  $\text{Ph}^1 (-)$  and  $\text{Ph}^1 (+)$  patients it may be useful to consider that they have different diseases.

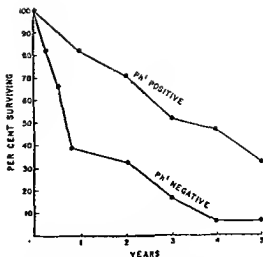


Fig. 48-7. Actuarial survival of 43 patients with and 18 patients without the  $\text{Ph}^1$  chromosome (From Erdinli et al.,<sup>28</sup> courtesy of the authors and Annals of Internal Medicine)

**CML IN CHILDREN.** Leukemia in infants and children generally is acute (Chapter 47); fewer than 5% of patients with CML are children.<sup>38,58</sup> Children and infants may have  $\text{Ph}^1 (+)$  CML,<sup>52,53,81,93,120</sup> in which case the clinical features of the disease, including response to therapy, are indistinguishable from those in adults.  $\text{Ph}^1 (+)$  CML has been reported in children as young as eight months of age.<sup>7</sup> However, infants more often have an atypical form of CML in which the  $\text{Ph}^1$  defect is not present. The course is less favorable in these infants than in those with  $\text{Ph}^1 (+)$  disease and clinical features mentioned below allow the disease to be distinguished from typical CML.<sup>7,22,52,55,81,93,110,120</sup> The  $\text{Ph}^1 (-)$  form of disease has been termed *juvenile CML*, but might better be referred to as *CML in infants* since its peak incidence appears to be at age one to two years.<sup>7,30,31,33,35,50,52,81,93,110</sup> Survival from the time of diagnosis usually is less than a year and response to therapy has been poor. The spleen is less large and lymphadenopathy is more common in these infants than in those with typical CML. An erythematous, desquamating skin rash is often present. Thrombocytopenia is frequently found when the diagnosis is made and leukocyte counts tend

to be lower than in typical CML. However, low LAP levels, eosinophilia, and basophilia are found, as in the  $Ph^1$  (+) form. In all the patients studied to date an increase in the proportion of fetal hemoglobin has been present, ranging as high as 55% of the total hemoglobin.<sup>7</sup> The persistence of fetal hemoglobin and the early age of onset of CML in infants as well as its development in infant siblings<sup>55</sup> have led to speculation that the disease may be a variety of congenital leukemia<sup>7,55</sup> (Chapter 47). Whether or not CML in infants is basically the same or a different disease from  $Ph^1$  (-) CML in adults will require further study.

## Morphologic Variants of CML

A diagnosis of any form of "eosinophilic" leukemia may be questioned (see also Chapter 47). However, there are certain patients in whom very striking eosinophilia is present and in whom an otherwise fairly typical picture of CML is found. To such disease the designation *chronic eosinophilic leukemia* might be applied. In most of these patients, increased numbers of mature and immature neutrophils are found in the blood in addition to the mature and immature eosinophils. Such findings may merely represent extreme instances of the less striking increase in eosinophils present in most patients with CML (page 1502). The  $Ph^1$  chromosome has been found in patients with chronic eosinophilic leukemia.<sup>45</sup> In some of the patients in whom it has been absent<sup>42</sup> the course of the disease has resembled that described as acute eosinophilic leukemia (page 1475).

A clinical picture of CML, but with extreme elevation in the basophil count has been observed (*chronic basophilic leukemia*).<sup>27,67,82,106</sup> Joachim<sup>60</sup> was the first to emphasize that extreme basophilia could be observed in a patient with otherwise typical CML. Practically all of the leukocytes may be basophils<sup>82</sup> or the absolute number of neutrophils may also be increased.<sup>27</sup> In some of the patients, eosinophilic and basophilic granules may be present in the same cell.<sup>27</sup>

The  $Ph^1$  chromosome may<sup>106</sup> or may not<sup>82</sup> be present. The validity of considering basophilic leukemia as a distinct form of CML has been challenged.<sup>67</sup> One of our patients had as many as  $80.0 \times 10^9/l$  basophils, but had an otherwise typical course for CML, responding well to busulfan and living for 87 months after the diagnosis had been made.

*Chronic monocytic leukemia* is a term sometimes applied to the condition in which there is marked elevation of monocytes in addition to the usual leukocyte changes noted in CML.<sup>27</sup> This term also has been applied to patients with modest monocytosis, who resemble patients with preleukemic leukemia<sup>77a</sup> (page 1477). Considering the evidence that neutrophils, eosinophils, monocytes, and, by inference, basophils share a common stem cell (Chapter 2) and that CML is probably a disease of that stem cell (Chapter 46) it is not surprising to find prominent eosinophilia, basophilia, or monocytosis rather than neutrophilia in a few CML patients.

*Chronic neutrophilic leukemia* (Emil Weil's CML with polynucleated neutrophils) is a term that has been applied to a condition in which few or no immature neutrophils have been found in the blood despite marked increases in mature neutrophils.<sup>83,100</sup> Survival of patients with this type of CML usually has been reported to be as long as or longer than that of patients with typical CML. The  $Ph^1$  chromosome, reported to date only in two patients, has been absent.<sup>69,100</sup>

## "Blast" Crisis

Blast crisis is the primary cause of death in CML patients and, simply stated, it represents the conversion of CML into an AML-like picture. In recently reported series, from 56<sup>61</sup> to 60%<sup>79a</sup> of patients with CML died in blast crisis; when those dying of causes unrelated to leukemia, drug toxicity, or undocumented causes were excluded, 84% of the remaining group were found to have died in blast crisis,<sup>61</sup> as did 86% in a British series.<sup>124</sup> Analysis of our own series likewise suggested that approximately 80% of

leukemia-related deaths were attributable to blast crisis. The incidence in other series ranges from similar to much lower figures.<sup>61</sup> Part of this variation may reflect differences in criteria for diagnosis of blast crisis.

**ONSET AND MANIFESTATIONS.** The time of onset of blast crisis in relation to duration of disease is plotted in Figure 48-8. Note that during the second through the sixth year this is basically a random phenomenon. A few patients first appear in blast crisis without a preceding period of symptomatic CML, as would be implied from the random nature of this event (Fig. 48-8). The onset of blast crisis may be quite insidious and, since busulfan is of little or no benefit once it has begun, recognition of the change is important. As the crisis develops, an increased percentage of blasts and promyelocytes is found in the blood and marrow and there may be a decrease in the absolute number of mature neutrophils in the blood. A rising leukocyte count or a rising absolute number of blasts and promyelocytes in the blood without a concurrent rise in mature neutrophils strongly suggests that blast crisis is beginning.<sup>15,32</sup> As noted previously (page 1502),

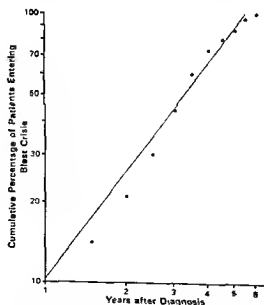


Fig 48-8 Time of onset of blast crisis after diagnosis. Note that the slope of the line is best described as an exponential function.

more than 20% blasts may be found at the time of diagnosis in patients not in blast crisis whose leukocyte counts are very high. However, if the number of blasts and promyelocytes together makes up 20% of the blood leukocytes in a previously treated patient, blast crisis almost always is under way.<sup>15,61</sup> The median percentages of myeloblasts and promyelocytes were 15% and 12%, respectively, in the blood, and 25% and 15%, respectively, in marrow, in patients whom we considered to be entering blast crisis.<sup>73</sup> In some patients, unexplained fever or thrombocytopenia not attributable to drug toxicity preceded increasing numbers of blasts in the blood. Other patients may be suspected to be entering blast crisis when anemia, splenomegaly, lymphadenopathy, or hepatomegaly develops out of proportion to what would be anticipated from the height of the leukocyte count. Using combinations of these findings, death within six months has been predicted with some accuracy.<sup>61</sup> Local blastic lesions of marrow that produced bone destruction have presaged the onset of systemic blast crisis.<sup>32</sup>

If blast crisis is suspected, but not evident from examination of the blood, the marrow should be examined. If blasts and promyelocytes exceed 30% of marrow cells in a patient with less than  $100 \times 10^9$  blood leukocytes/l, then blast crisis can be diagnosed with some confidence. Additional information may be gained from direct chromosome analysis of the marrow or blood (Chapter 46).

*Extramedullary blast crisis* has been reported.<sup>19,35,87</sup> In these patients, myeloblastic tumors in locations such as the breast, meninges, or lymph nodes developed before the blood and marrow were predominantly blastic. Unless imprints are examined, incorrect diagnoses of other types of tumors such as reticulum cell sarcoma<sup>35,87</sup> may be made on sections of these blastic tumors. However, it is also possible that a reticulum cell sarcoma-like disease may complicate CML. The difficulties relating to the differentiation of myeloblastic and reticulum cell tumors have been discussed in depth, but without clear resolution.<sup>83</sup>



**CHROMOSOME ABNORMALITIES.** The majority of patients entering blast crisis are found to have chromosome abnormalities in addition to their previously demonstrated  $Ph^1$  abnormality (Chapter 46).<sup>4,15,102,120</sup> However, not all such abnormalities are indicative of the onset of blast crisis. Additional G group chromosomes, including a duplicated  $Ph^1$  chromosome, have been present for years in some patients with typical CML.<sup>102</sup> Certain others have been shown to have a duplicate  $Ph^1$  appear during blast crisis, disappear with remission, and reappear as blast crisis once again supervenes.<sup>112</sup> An attempt was made to determine which, if any, additional chromosome abnormalities might be associated with blast crisis by serial studies of a group of 27 patients, all of whom had a  $Ph^1$  defect.<sup>89</sup> The percentage of cells with chromosome defects in addition to  $Ph^1$  and the type of defect were correlated with the percentage of immature cells in the blood (Fig. 48-9A, B, and C). There was a significant and positive correlation between the percentage of hyperdiploid cells and the percentage of immature cells (Fig. 48-9A and B). However, pseudohypodiploid cells were not positively correlated with immaturity (Fig. 48-9C). Pseudohypodiploid cells are those with supranumerary chromosomes, but with deletion of other chromosomes exceeding the additions so that 45 or fewer chromosomes are present. Deletion of C group chromosomes was observed most commonly. These findings indicate that certain chromosome additions may be associated with blast crisis, but also suggest that deletion of certain chromosomes, particularly C group chromosomes, may protect the patient from developing blast crisis. In that regard the favorable prognosis associated with deletion of the Y chromosome (page 1509) is of interest.<sup>36</sup> Ob-

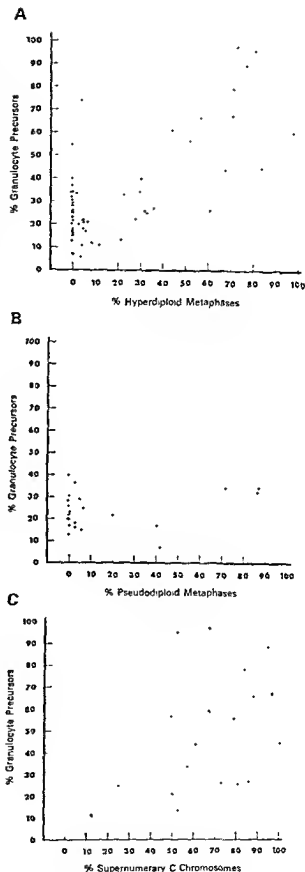


Fig. 48-9. Relation of chromosome abnormalities, in addition to the  $Ph^1$  abnormality, to the immaturity of blood cells in CML. A, Relation of the percentage of myeloblasts, promyelocytes, and, myelocytes to hyperdiploid metaphases, B, to pseudodiploid metaphases, and, C, to supernumerary C group chromosomes (From Pederson,<sup>89</sup> courtesy of the author and Scandinavian Journal of Haematology)

viously, more studies of specific chromosome defects, additions, and deletions are necessary before their role in blast crisis can be defined.

**COURSE** As the blast crisis deepens, neutropenia develops and bacterial infections become a problem. Thrombocytopenia occurs and hemorrhagic phenomena appear. Anemia becomes increasingly severe and transfusion may be necessary. The spleen may enlarge rapidly. Hepatomegaly and lymphadenopathy as well as infiltration of other organs may develop. Extremely large lymph nodes may be found. Fever without evident infection is common.

**DIFFERENTIATION FROM ACUTE LEUKEMIA.** Thus, all of the common features of AML develop and yet there are features that allow one to distinguish blast crisis of CML from AML even if a history of known CML is lacking. Basophilia commonly persists and may increase during blast crisis, but is uncommon in AML. Eosinophilia usually disappears. In most patients there is a greater percentage of persisting intermediate cells such as myelocytes than in AML. Spleen size is larger in patients having blast crisis than in those with AML. In 95% of our patients with AML the spleen was less than 10 cm below the costal margin when the diagnosis was made, but splenomegaly of this degree was present in 70% of patients with CML entering blast crisis.<sup>75</sup> The LAP score is not particularly helpful since it may increase in blast crisis<sup>15,102</sup> and it may be abnormally low in AML (Chapter 47). If the Ph<sup>1</sup> chromosome is present, blast crisis rather than AML is suggested strongly.

**CAUSE.** The cause of blast crisis is unknown. It has been suggested from its relative infrequency in older series<sup>59</sup> that it is more common in patients treated with busulfan than in those treated by other means. However, it is uncertain that the cause of death in earlier reported patients was examined as closely as in patients reported more recently. The frequency with which new cell lines identifiable by chromosome abnormalities appear suggests that blast crisis represents clonal evolution of a more malignant cell

line.<sup>15,102,111,112</sup> This suggestion gains additional support from reports of patients in whom new chromosome abnormalities appeared with blast crisis, disappeared during an induced remission, and reappeared with return of blast crisis.<sup>11,20,37,90,112</sup> The evolution of blast crisis in apparently single, extramedullary sites<sup>19,35,87</sup> or in isolated areas of marrow<sup>32</sup> is compatible with a clonal evolution. However, explanations other than clonal evolution of a malignant cell line can be found for chromosomal change in leukemia<sup>102</sup> (see Chapter 46).

The morphologic characteristics of the immature cells in blast crisis of CML generally has been compatible with a myeloblastic evolution. However, on occasion, an *erythroblastic evolution*, reminiscent of DiGuglielmo's syndrome (Chapter 47), has been observed.<sup>112</sup> We also have observed patients in whom the blastic evolution was evidenced by immature cells predominantly of the morphologic type best described as monocytic or myelomonocytic (Chapter 47). This type of evolution (erythroblastic or monocytic) is compatible with our current concept of the nature of CML; namely, it is a disease affecting stem cells potentially pluripotent for mature neutrophilic, monocytic, and erythrocytic as well as eosinophilic and basophilic cells (Chapter 46).

**THERAPY.** Therapy of blast crisis is no more satisfactory than that of AML (Chapter 47) and perhaps even less so. Combined chemotherapy with 6-MP, methotrexate, vincristine, and prednisone induced a smaller number of remissions in patients with blast crisis of CML than in those with AML.<sup>30</sup> In general the same drugs that are effective in AML subjects have induced occasional remissions in patients with blast crisis of CML. We observed five complete remissions in 11 patients treated with combined 6-mercaptopurine and cytosine arabinoside, but these were of short duration, averaging only five months. Remission also has been reported in nine of 30 patients treated with vincristine and prednisone, agents which are rarely, if ever, of benefit in AML, although useful in ALL.<sup>15</sup>

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## Chronic Lymphocytic Leukemia

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CHRONIC lymphocytic leukemia (CLL) is characterized by an excessive number of small lymphocytes in the blood and bone marrow. In most of the patients, excessive numbers of lymphocytes also are found in lymph nodes, spleen, liver, and other organs. The disease is unusual in persons younger than 30 years, becomes increasingly frequent with increasing age, and is approximately twice as frequent in males as in females (Chapter 46). Reports describing children with the disease in its characteristic form are exceedingly rare.<sup>12,28-29b</sup>

Chronic lymphocytic leukemia behaves less like a "cancer" than do any of the other diseases discussed in this section. Actually the use of the term "leukemia," from the standpoint of its impact on the patient, is unfortunate. The term "leukemia" is used to refer to disorders that are much more serious from the standpoint of morbidity and mortality

than is chronic lymphocytic leukemia. As discussed in Chapter 46, it is true that there is suggestive evidence that CLL represents the abnormal growth of a clone of "B"-type lymphocytes and thus, in that sense, it is a tumor. The lymphocytes accumulate in a variety of tissues and organs but do not produce detectable functional damage in most subjects. The prolonged survival and the failure of the survival curve to fit a log probability distribution,<sup>7</sup> however, are in contrast to most cancers. Many of the major complications reflect immunologic aberrancy. In 1914, Moreschi<sup>11</sup> noted that antibodies directed against the typhoid bacillus failed to appear in the serum of a patient with typhoid fever who also had CLL. Subsequent studies have suggested that failure to form circulating antibody is the primary cause of the frequent and severe infections observed in patients with CLL (Chapter 54).

It has been suggested<sup>18-23,56</sup> that there is a *benign form* and a *malignant form* of CLL, but studies indicate that in patients whose course seemed to be more rapid than in those who lived long following diagnosis, the diagnosis was probably made at a later stage of disease.<sup>7</sup>

### Mode of Presentation<sup>7,23,57</sup>

Approximately 25% of patients have no symptoms attributable to CLL at the time the diagnosis is made. These patients are found

to have blood lymphocytosis or enlarged lymph nodes or spleen on routine examination or during evaluation of apparently unrelated diseases. As with other forms of leukemia (Chapters 47 and 48) the most frequent complaint that causes the patient to consult a physician is fatigue or a sense of lack of well-being (Table 49-1). Less frequently, enlarged nodes or the development of an infection is the initial complaint (Table 49-1). Rarely, there are complaints of easy bruising or other bleeding problems. Weight loss is noted by some patients but this is rarely severe. Fever, in the absence of infection, rarely, if ever,<sup>6</sup> occurs in patients with CLL, in contrast to its frequent occurrence in those with the acute leukemias or the lymphomas (Chapter 54).

## Physical Findings

Enlarged lymph nodes are found in most patients, as is splenomegaly (Table 49-1). Hepatomegaly is detected in half of the patients, but sternal tenderness is much less frequent than in those with other forms of

leukemia (Table 49-1, Chapters 47 and 48). Lymph nodes usually are discrete, freely movable, and nontender, as in most forms of leukemia and lymphoma. Cervical and supraclavicular nodes are the ones most commonly enlarged, but palpably enlarged nodes in cervical, axillary, and inguinal areas also are detected in approximately half the patients at the time of diagnosis.<sup>7</sup> Splenomegaly usually is modest. The spleen extended less than 10 cm below the costal margin in 90% of our patients. It is firm and splenic infarction is much less common than in patients with CML or the acute leukemias. Less common manifestations observed in our series<sup>7</sup> were infiltration of tonsils (7%), masses presumed to be retroperitoneal nodes (2%), and skin infiltration (2%). Enlarged hilar lymph nodes or a widened mediastinum were detected on chest x ray in 5%. Pallor and ancillary signs of anemia rarely are prominent since anemia usually is mild at the time of diagnosis (page 1523). Similarly, because thrombocytopenia usually is mild, if present, petechiae or ecchymoses were noted in only 5%. Bacterial infection, most often pneumonia, with the

**Table 49-1. Signs, Symptoms and Blood Values in CLL at the Time of Diagnosis**

	Patients Surviving		All Patients
	Less than 5 yrs	More than 5 yrs	
Number of patients	35	41	130
Mean age (years)	61.2	56.8	59.5
Symptoms (percent of patients)	94	60	76
Fatigue* †	80	40	60
Noted masses	52	20	39
Infection	30	5	25
Signs (percent of patients)	100	91	95
Lymphadenopathy	88	87	83
Splenomegaly	92	69	74
Hepatomegaly	56	27	45
Sternal tenderness	43	17	27
Blood			
Lymphocytosis, mean $\times 10^9/l$	119.0	55.0	96.0
More than 100 lymphocytes $\times 10^9/l$ (percent of patients)	45	10	32
Anemia (percent of patients)	85	28	55
Thrombocytopenia (percent of patients)	57	29	39
Neutropenia (percent of patients) ‡	27	6	17

\*Fatigue, malaise or a vague sense of ill health

†Figure is probably exaggerated due to error in counting a minor cell population with a 200 cell differential count (Adapted from Boggs et al.<sup>7</sup>)

expected attendant signs, is a fairly frequent finding. A few patients, 5% of our series,<sup>7</sup> have no detectable physical abnormalities and the majority of such patients also are asymptomatic.

## Laboratory Findings

*Blood lymphocytes* do not exceed  $4.5 \times 10^9/l$  in normal subjects (Chapter 6). Patients with CLL may have as many as  $1000 \times 10^9/l$ , but the majority have less than  $100 \times 10^9/l$  when the diagnosis is made (Table 49-1), in contrast to those with CML in whom higher counts are usually found (Chapter 48). The exact level of blood lymphocyte

count at which one should consider the diagnosis of CLL is difficult to establish. When there has been opportunity to follow the development of CLL, the rise in blood lymphocytes has been very gradual but more or less continuous in the early stages (Fig. 49-1).<sup>20</sup> Cyclic fluctuations in numbers of blood lymphocytes may occur in untreated patients<sup>23</sup> and these may be very substantial, the count decreasing by as much as  $50 \times 10^9/l$  between visits to the physician. In certain untreated patients the lymphocyte count eventually becomes stabilized and may not rise further during months or years of observation.<sup>23</sup>

In most patients the average lymphocyte

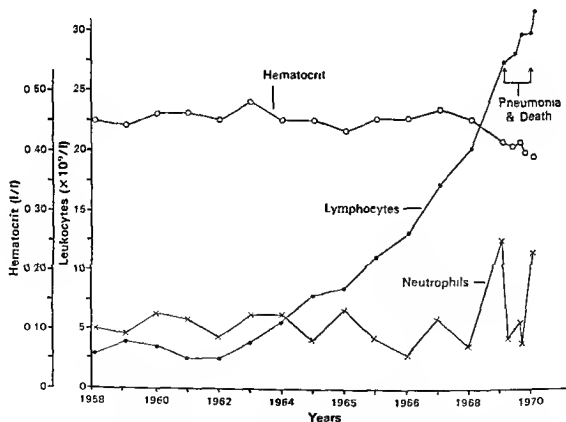


Fig 49-1 The patient a 72 year old man had been hospitalized, because of psychiatric problems, at a Veterans Administration Hospital for 27 years when hematologic consultation was sought because of pneumonia and lymphocytosis. A routine hematocrit determination and leukocyte and differential counts had been done at yearly intervals. The number of blood lymphocytes had begun to increase eight years before the diagnosis of CLL was made at the time of the consultation. Small but enlarged cervical nodes and a palpable spleen tip were the only physical abnormalities other than the signs of pneumonia. Serum gamma globulin was abnormally low 0.4 g/dl. One year after the diagnosis of CLL was made the patient died from severe recurrent pneumonia.



has the *morphologic appearance* of the small lymphocyte seen in normal blood (Plate XXI). A minor population of abnormal-appearing lymphocytes often is present and in a few patients the average lymphocyte may be larger than normal and appear somewhat immature. Cells with deeply clefted nuclei (*Reiðer cells*) may predominate<sup>11</sup> and when the lymphocytes are abnormal it may be difficult to distinguish CLL from lymphosarcoma invading the blood or from leukemic reticuloendotheliosis (see Chapter 51). Cytoplasmic inclusions may be observed in lymphocytes from patients with CLL. In one study, crystalline inclusions that proved to be intimately associated with IgM  $\lambda$  were found in cells from four of 30 patients.<sup>15a</sup> Inclusions appearing as clear, geometric areas in Wright-stained smears that were made up of fibrillar bundles of unknown material have been noted,<sup>41a</sup> as have other globular, tubular, or rod-shaped inclusions.<sup>15a</sup> Some large inclusions have some of the characteristics of polyribosomes.<sup>1</sup> Normal lymphocytes may contain organelles that are membrane bound and stain positively for acid phosphatase, thus having characteristics of lysosomes; such structures are less frequent in lymphocytes from patients with CLL than in normal blood.<sup>18a</sup>

We were unable to correlate the frequency of unusual-appearing lymphocytes with any change in the clinical features of CLL in our series,<sup>7</sup> although others have suggested a more rapid course when numerous abnormal lymphocytes are present.<sup>3,54</sup> Ruptured leukocytes (basket cells) are common on blood smears of most patients with CLL but a satisfactory explanation for their presence has not been offered.

*Anemia* was present when the diagnosis was made in half of our patients (Table 49-1), but was usually mild, the *VPRC* being below 0.301/l in only 7% of the patients. The anemia usually is normochromic and normocytic and reticulocytes are normal or decreased in number. On occasion, severe hemolytic anemia is present at the time of diagnosis.<sup>1a,57</sup> Platelets are normal in number in more than half the patients (Table 49-1), and when

*thrombocytopenia* is present it is usually mild, platelet counts of less than  $50 \times 10^9/l$  being uncommon. *Neutrophil concentration* usually is normal, averaging  $6.0 \times 10^9/l$  in our series<sup>7</sup>; the incidence of neutropenia is probably overestimated in Table 49-1 by virtue of the distributional error for a minor leukocyte population in differential counts.

*Bone marrow aspiration or biopsy* is of little or no help in making a diagnosis of CLL when striking degrees of blood lymphocytosis are present. If blood lymphocytes are increased only to a slight degree, eg, 5.0 to  $10.0^9/l$ , the finding of disproportionately increased numbers in bone marrow may provide some diagnostic support. However, almost all patients with symptomatic CLL have in excess of  $10.0 \times 10^9/l$  when the diagnosis is made.<sup>7</sup> In patients with palpably enlarged lymphoid tissue but without blood lymphocytosis, marrow examination may disclose lymphosarcoma (Chapter 51). Biopsy discloses a hypercellular specimen with either focal or diffuse infiltration with lymphocytes. It must be kept in mind that smears of aspirated marrow also contain cells from the blood so that the percentage of marrow lymphocytes may be overestimated if blood lymphocyte counts are high. Rarely, normal percentages of marrow lymphocytes have been noted when blood lymphocytosis was present.<sup>57</sup>

Serum *uric acid* usually is normal and uric acid excretion rates often are normal.<sup>53</sup> *Immunoglobulin levels* are decreased in many patients (pages 1525 and 1526). Serum *albumin* and alpha and beta globulin levels usually are within normal limits.<sup>5</sup> Serum electrophoresis on acrylamide gel, a procedure yielding some 25 separate protein bands in normal sera, reveals the loss or marked decrease of a normal band, the "P" band, which moves near transferrin, and also shows a new "X" band which moves even closer to transferrin than "P."<sup>65</sup> The significance and nature of this change must await further study, but the absence of a similar change in lymphosarcoma (LSa) suggests that this may be a specific finding in CLL. Excessive excretion of urinary pseudouridine is present in most patients with CLL.<sup>64</sup>

## Differential Diagnosis

Diagnostic problems are uncommon in CLL since other causes of prolonged lymphocytosis are rare. Recovery phases of infection, especially chronic infections such as tuberculosis, may be accompanied by modest degrees of lymphocytosis.<sup>25,47,68</sup> Transient, but fairly profound increases in small lymphocytes in the blood may accompany pertussis or infectious lymphocytosis, but these usually are diseases of children or young adults. Striking lymphocytosis was reported in a patient with pernicious anemia, thought not to be due to coexistent CLL.<sup>2</sup> As reported by Sagoe,<sup>52</sup> "tropical splenomegaly" (page 1411) may mimic CLL to a greater extent than any other disease. This disease of unknown cause responds to therapy with proguanil, an antimalarial compound. Sagoe reported a series of patients with seemingly identical signs and symptoms, some of whom responded to proguanil therapy but others did not. Three of 11 nonresponders eventually developed typical CLL. In responders blood lymphocytosis was as high as  $35.0 \times 10^9/l$  and there was lymphocytic infiltration of the liver and marked splenomegaly. Responders differed from nonresponders in IgM levels, which were high in the former and low in the latter, and in lymphocyte blastogenic response to phytohemagglutinin, which was normal in responders but abnormal in 10 of 11 nonresponders.

Infectious mononucleosis and other viral illnesses that produce an infectious mononucleosis-like blood picture (Chapter 43) usually are found in children or young adults and should be easily distinguished from CLL by the morphologic appearance of the cells and the attendant signs and symptoms.

Basically, if lymphocytosis persists for some months in a middle-aged or elderly patient in whom no symptoms and signs are present or in whom symptoms and signs suggest CLL, the diagnosis can be made. However, since therapy at this stage is probably ill advised (page 1529) there is no urgency in making a firm diagnosis. Biopsy of enlarged

lymph nodes, marrow examination, or other histologic study may or may not reassure the physician in the correctness of his diagnosis but are unnecessary and do not benefit the patient.

## Variants of CLL

Most physicians will not make the diagnosis of CLL unless an excessive number of lymphocytes is present in the patient's blood. Others recognize an "aleukemic" form in which excessive lymphocytes are present in marrow but not in the blood.<sup>23</sup> This form is perhaps better designated as *lymphocytic lymphosarcoma (LSa) of bone marrow* (Chapter 51). There is evidence that the prognosis of such patients is inferior to that of patients with CLL.<sup>7,48</sup> For example, among patients with "CLL" whose leukocyte counts were less than  $10.0 \times 10^9/l$  at the time of diagnosis, 5-year survival was less than half that observed in the entire group.<sup>70</sup> The pathologic changes in lymph nodes, spleen, and liver appear identical in CLL and in lymphocytic LSa.<sup>20,45</sup>

Patients may have skin infiltration when first seen (Fig. 49-2) and later develop lymphocytosis, a condition that has been referred to as *erythrodermia* or *Sézary's syndrome* (Chapter 51). Also, a CLL-like blood picture may develop during the course of lymphosarcoma; this is termed *leukolymphosarcoma* (Chapter 51). A few patients with otherwise typical ALL have moderately mature-looking lymphocytes (Chapter 47). Rarely, abnormal lymphocytic manifestations defy classification. We observed one man, 62 years of age, who had large lymphoblastic-appearing cells in his blood (Fig. 49-3), with counts ranging as high as  $300.0 \times 10^9/l$  for at least 5½ years and possibly even twice that long. This patient had none of the usual features of ALL and none of the immunologic deficits or aberrancies of CLL.

## Course of Disease and Cause of Death

As evidenced by the very prolonged survival that is possible in patients with CLL

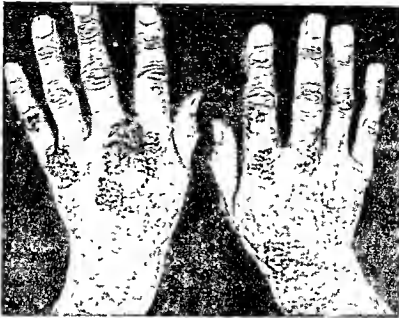


Fig. 49-2. Skin lesions in a patient with chronic lymphocytic leukemia, with an inflammatory reaction superimposed on leukemia cuts. On a base of indurated skin there is a brownish encrustation due to the oozing of a pink fluid. There was marked general glandular enlargement, splenomegaly, and a leukocytosis of  $200 \times 10^9/l$  with 93% lymphocytes.

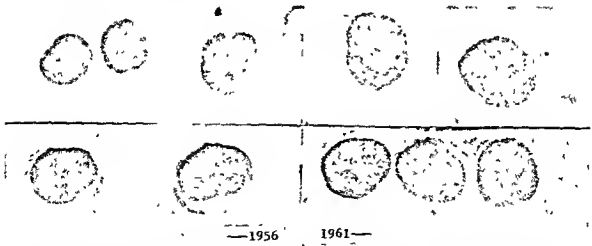


Fig. 49-3. Blood cells of a patient with "chronic lymphoblastic leukemia." The lymphoblasts were present continuously in large numbers in the blood for at least 5½ and perhaps 11 years before the patient (CM 5374) died in 1962. An increased leukocyte count had been noted in 1951 at a time when the patient suffered from fatigue and foot drop. In 1956, fever of unknown cause developed and lymphoblasts were found in the blood smear and were noted on all subsequent examinations until the patient's death. Exploratory laparotomy with liver and lymph node biopsy and splenectomy showed little abnormality and while clumps of blasts were present on aspiration of marrow, abundant normal cells were also present. Lymphadenopathy never developed but when more than  $300 \times 10^9$  lymphoblasts/l were present, moderate anemia and thrombocytopenia were noted. At this stage, sequential therapeutic trials of prednisone, 6-MP, and methotrexate were of no benefit. The patient died at home of unknown causes at age 67. Since his course failed to fit into the syndromes of either ALL, CLL, or lymphosarcoma the diagnosis "chronic lymphoblastic leukemia" was made.

(page 1527) the course often is quite benign and many patients remain asymptomatic for years.<sup>7,67</sup> Since this is a disease of the elderly, unrelated but coexisting disease is a relatively common cause of death (Table 49-2).<sup>7,20b,57</sup> Death attributable to CLL usually is caused by uncontrolled infection<sup>7,20b</sup> (Table 49-2). Unless myelotoxic therapy has induced neutropenia, infection is usually attributable to deficient antibody production (page 1520, Chapters 46 and 54). Patients without extensive evidence of disease may have hypogammaglobulinemia (Fig. 49-1), and development of infection may be the first evidence of disease (Table 49-1); without vigorous and appropriate antibiotic therapy the outcome may be fatal. Because of susceptibility to infection, vaccination with living organisms, such as smallpox, is contraindicated (Chapter 54). Since fever due to the disease itself is exceedingly rare<sup>6,7</sup> the appearance of fever in persons with CLL should be assumed to indicate the presence of infection. It has been stated, without documentation, that fever occurs due to disease in "aggressive" CLL, especially when retroperitoneal nodes are present.<sup>51</sup> This has not been our experience; the possibility of obscure infection may be difficult to rule out. In general, the antibody deficit and the frequency of infections increase with duration of disease (Chapter 54).

*Transformation into an acute phase*, the usual cause of death in CML (Chapter 48), is exceedingly rare in CLL, if it ever occurs. Transition from a picture of LSA to CLL and then to ALL is occasionally observed (Chapter 51), but we have never encountered a patient with an initial diagnosis of CLL who developed an ALL-like picture. There are a few reports<sup>38,39</sup> suggesting such a transformation, but the cases of CLL usually have been atypical.<sup>38</sup> Other reports<sup>43</sup> suggest coexistence of two diseases rather than transition. A search of 340 cases of CLL revealed a terminal picture of acute leukemia in two patients,<sup>39</sup> one AML and one ALL. This was slightly but not significantly above the expected frequency of acute leukemia in the general population. There are at least two

**Table 49-2. Cause of Death in 50 Patients with CLL**

	Percent
Death apparently related to CLL	54
Due to infection	46
Due to thrombocytopenia	4
Meningeal infiltration and meningitis	2
Hemolytic anemia	2
Death related to complications of therapy*	16
Death apparently unrelated to CLL†	30

\*Hemorrhage or infection during development of profound thrombocytopenia and/or neutropenia following therapy with nitrogen mustard (4 patients) urethane (1 patient) or chlorambucil (1 patient) serum hepatitis (1 patient) and acute tubular necrosis following transfusion (1 patient).

†Myocardial infarction (3 patients) congestive heart failure without anemia (2 patients) thrombophlebitis and pulmonary embolism (2 patients), diabetes (2 patients) and one each with prostatic carcinoma bile duct carcinoma, Hodgkin's disease, perforated peptic ulcer, cerebral thrombosis, and an auto accident (Adapted from Boggs et al.<sup>7</sup>)

reports of AML developing in patients with CLL after prolonged therapy with chlorambucil as well as at least 17 after <sup>32</sup>P therapy.<sup>43</sup> These developments are thought to be related to the leukemogenic effects of the therapeutic agents used, rather than to natural transitions. One of our patients developed Hodgkin's disease, an occurrence also reported by others,<sup>64</sup> but whether this was due to chance or was a meaningful association cannot be determined.

*Inappropriate antibody formation* appears to be responsible for many serious complications in CLL. Coombs'-positive hemolytic anemia of severe degree complicates the course of from 10 to 20% of patients<sup>57</sup> and a platelet consumption syndrome similar in its manifestations to that of autoimmune thrombocytopenia (Chapter 34) is seen in a few.<sup>7,19</sup> The diagnosis and management of these complications are discussed in Chapter 54. Other bizarre immune responses, such as exaggerated delayed hypersensitivity reactions to mosquito bites, may develop.<sup>63</sup> The appearance of cryoglobulinemia with symptomatic cold urticaria<sup>16</sup> or of cryofibrinogenemia<sup>32</sup> during the course of CLL might

be included in this category. Monoclonal serum protein spikes may be observed in as many as 5% of patients with CLL<sup>12,50</sup> and a fairly typical picture of myeloma including marked marrow plasmacytosis and bone lesions has been a terminal complication in patients observed by us and others.<sup>61</sup>

*Carcinoma* is considered by some<sup>57</sup> to be more common in CLL patients than would be expected on statistical grounds, but other series in which the incidence in association with CLL was compared with age-matched controls did not disclose an excess occurrence of other tumors (Chapter 46).

Severe anemia, thrombocytopenia, and/or neutropenia, presumably due to *marrow failure* since no evidence of excessive cellular destruction is present, may develop eventually.

### Duration of Survival

The average patient in our series lived for six years after the diagnosis had been made, as calculated by actuarial survival methods (Fig. 49-4).<sup>7</sup> These survival figures are virtually identical to those noted by Osgood,<sup>42</sup> slightly lower than the selected series of Hill et al.<sup>30</sup> and probably not significantly higher than those reported in certain other relatively recently reported series.<sup>27,29b,31</sup> Zippin et al.<sup>70</sup> found a median survival of slightly more than four years in 839 patients from 24 hospitals in whom the diagnosis was made between 1955 and 1964. In older series,<sup>4,35,40,60</sup> shorter average survival was reported, but in these studies patients who were still alive usually were excluded. Analysis of our series in the same manner that Bethell<sup>4</sup> and Leavell<sup>35</sup> utilized in analyzing theirs revealed a slightly but not statistically significant longer survival in patients treated between 1945 and 1964 as compared to those treated before 1940.<sup>7</sup> Direct comparison of survival in various series has little meaning, however, because of the influence of uncontrolled selective factors. Not only does treatment differ from series to series but the population seen by a referral center may differ from that of a general hospital. For example, the series of

patients analyzed by Zippin et al.<sup>70</sup> tended to be older than our own.<sup>7</sup> While exact figures cannot be given it seems likely that average survival from diagnosis of CLL is from four to six years.

Very prolonged survival is possible. The longest survivor in our series died of pneumonia at age 79, 24 years after a diagnosis of CLL was made. Survival for as long as 35 years has been reported.<sup>44</sup> Since many patients die of unrelated causes (Table 49-2) the course of CLL is more accurately reflected in survival curves that exclude death from unrelated causes (Fig. 49-4)—“disease-oriented” versus “patient-oriented” survival.<sup>7</sup> When so calculated, median survival from the time of diagnosis was nine years in our patients.<sup>7</sup>

The duration of survival in part depends upon the severity of the disease at the time of diagnosis. Patients who survived for more than five years had less severe signs and symptoms and less abnormal laboratory findings than did those who survived for less than five years (Table 49-1). However, since members of the latter group were older and had had symptoms for a longer period before diagnosis (Table 49-1) than did the longer survivors it appeared to us that the more severe manifestations at the time of diagnosis reflected a longer period of preexisting, unrecognized disease rather than the existence of more “aggressive” disease.<sup>7</sup> Specific factors may be correlated with duration of survival. Young patients developing CLL tend to live longer after the diagnosis is made than older ones,<sup>7,35,42,70</sup> in contrast to the clinical impression of some.<sup>18</sup> Females may live longer than males.<sup>42,70</sup> An inverse relationship between the height of the leukocyte count and duration of survival was found in some<sup>7,35</sup> but not all<sup>4,42,57</sup> studies. In one study, patients with leukocyte counts of less than  $25.0 \times 10^9/l$  or of more than  $50.0 \times 10^9/l$  survived for a shorter time than did those whose initial count was between these two figures.<sup>70</sup> Anemia<sup>7,35</sup> and thrombocytopenia<sup>7</sup> are poor prognostic signs. Actually, although specific correlations are poor, there is a direct relationship between survival and

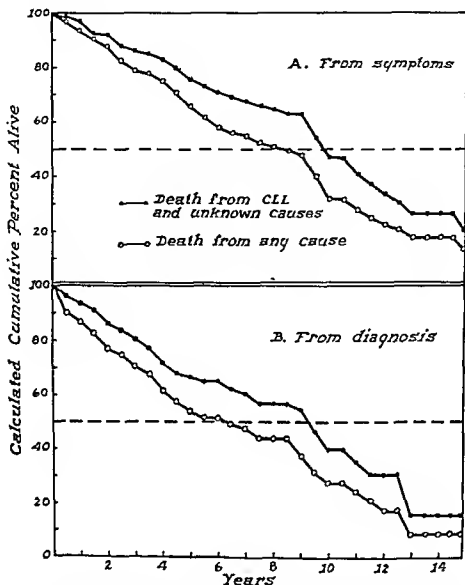


Fig 49-4 Survival of 130 patients with chronic lymphocytic leukemia from onset of symptoms (A) or from time of diagnosis (B), based on deaths from all causes and deaths related to the leukemia (From Boggs et al \* courtesy of the authors and American Journal of Medicine)

the presence of symptoms, signs, and abnormal laboratory findings in patients with CLL.<sup>7,23,57,70</sup> As shown in Table 49-1, patients surviving for the shortest time had more severe disease as judged by any parameter than did those surviving for longer periods. However, certain complications, such as hemolytic anemia<sup>37</sup> or skin infiltration,<sup>35</sup> may have no prognostic significance if proper treatment is given. Scoring methods to pre-

dict survival have been suggested.<sup>49</sup> In general, in the aggregate the more evidence of disease at the time of diagnosis, the shorter is the survival time. Nevertheless, individual variation is so great that such predictions are of little individual benefit. We have observed patients who, when first examined, had extensive disease, including anemia and thrombocytopenia, and lived more than 10 years. Others with minimal evidence of disease died

in less than five years of recurrent infection.

If those who have minimal disease when first seen by the physician are patients in whom the diagnosis is made at an early stage rather than those having a benign form of disease, then the survival of those in whom the diagnosis was made before symptoms developed may more accurately reflect the true course of the disease. Members of such a group may live for an average of at least 10 years (Fig. 49-5).

## Therapy

### Principles of Management

Whether antileukemic therapy favorably influences survival is moot. The slight increase in survival of our patients,<sup>7</sup> who were treated primarily with alkylating agents and

steroids or not at all, and of those of Osgood,<sup>42</sup> who were treated with  $^{32}\text{P}$ , as compared to Leavell's<sup>35</sup> and Bethell's<sup>4</sup> patients, who were treated primarily by x-irradiation, easily could be accounted for by the availability of therapy for infection and hemolytic anemia in our and in Osgood's series. Survival figures for a large untreated series are not available, but in 1924 Minot and Isaacs<sup>40</sup> reported no difference in survival of a small group treated by irradiation as compared to an untreated group. Osgood's patients<sup>42</sup> received more vigorous therapy than did ours,<sup>7</sup> suggesting that vigorous antileukemic therapy may not prolong survival. In a controlled trial, patients treated with  $^{32}\text{P}$  lived for a shorter period than did those treated with chlorambucil or triethylenemelamine.<sup>31</sup> However, the survival period of this  $^{32}\text{P}$ -treated group was significantly shorter

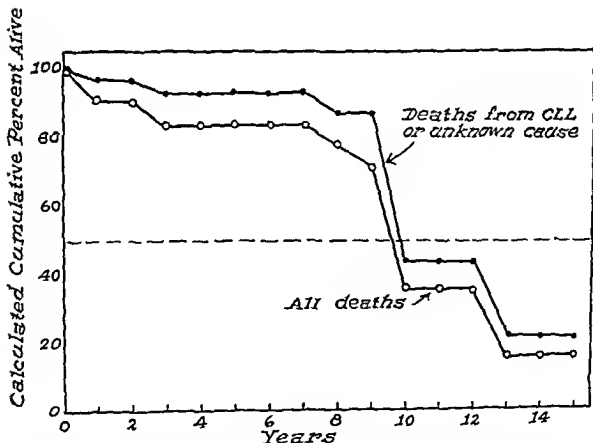


Fig 49-5 Survival of 31 patients in whom the diagnosis of CLL was made before symptoms attributable to the disease had developed (From Boggs et al,<sup>7</sup> courtesy of the authors and American Journal of Medicine)

than that of the group treated with  $^{32}\text{P}$  by Osgood<sup>42</sup> and the remainder survived no longer than did members of other series.<sup>7,42</sup> Thus, the correct conclusion from Huguley's study<sup>31</sup> may be that  $^{32}\text{P}$  shortened life rather than that life was prolonged by chlorambucil or triethylenemelamine. We cannot agree with the conclusion reached by some<sup>50</sup> that life clearly is prolonged by therapy and that therapy should be employed routinely in most patients. In an uncontrolled study, longer survival was noted in patients who were not treated within four months of diagnosis, as compared with those who were.<sup>70</sup>

If, as suggested, antileukemic therapy fails to prolong survival, then the use of such therapy must be examined with respect to its effects upon morbidity. The proper use of adjunctive therapy in patients with complications (Chapter 54), especially infection, hemolytic anemia, and obstructive masses, may be more important than systemic antileukemic therapy. Certainly one cannot favorably influence morbidity in asymptomatic patients; consequently, it is our practice to observe such patients as well as those with minimal symptoms and give no therapy. In contrast to the numerous controlled studies of therapy, including comparison of antileukemic drugs and placebos, which have been carried out in acute leukemia (Chapter 47), reports of studies that systematically compare the effect of routine antileukemic therapy to watchful waiting in CLL have not as yet been published. Consequently, indications for therapy are difficult to define unless complications such as hemolytic anemia (Chapter 54) are present. However, a trial of therapy is certainly in order if symptoms become increasingly troublesome due to increasing anemia or perhaps due to the increased metabolic demands of large tumor masses.

If treatment is decided upon, several forms of therapy are available.

#### Steroids

Virtually all patients who are treated with 0.5 to 1.0 mg of prednisone per kilogram of

Table 49-3. Effect of Therapy on CLL

	Favorable Effect (%)	Unfavorable Effect <sup>*</sup> (%)
<b>Chlorambucil or triethyl- enemelamine (100 courses)</b>		
Blood lymphocytosis	80	0
Lymph node, liver and spleen size	55	10
Anemia	14	23
Thrombocytopenia	4	4
Neutropenia	2	20
<b>Steroids (29 courses)</b>		
Blood lymphocytosis	17	0
Lymph node, liver and spleen size	68	0
Anemia	83	0
Thrombocytopenia	48	0

<sup>\*</sup>Enlargement of nodes or other tissues during therapy or reduction of red cells, platelets, or neutrophils persisting for more than six weeks after therapy was stopped (Adapted from Boggs et al.<sup>71</sup>)

body weight per day report significant symptomatic improvement.<sup>7,55</sup> Furthermore, significant regression of the size of lymph nodes and of the spleen as well as lessening of anemia and thrombocytopenia usually are observed (Table 49-3). In patients receiving steroid therapy, as lymph nodes and spleen decrease in size the blood lymphocyte concentration usually increases, doubling and even tripling from pretreatment values.<sup>55</sup> However, after some weeks of therapy, lymphocyte concentration begins to fall and may then decline to below pretreatment values.<sup>7,55</sup> Thus, from examination of subjective and objective antileukemic effects, prednisone would appear to be an excellent drug. However, in a controlled, "cross-over" type of study the frequency of life-threatening infections in patients with CLL was suggestively higher during a three-month period of prednisone therapy as compared to a three-month control period.<sup>53</sup> Since infection is the major cause of death and since infections in steroid-treated patients with a variety of diseases are unusually severe, this side effect of prednisone limits its usefulness in patients with CLL. Nevertheless, when severe anemia



requiring regular transfusion develops even in the absence of hemolysis, response to steroids may be noted and long-term therapy may be justified despite the risk of infection.<sup>22</sup> In some patients this form of anemia also responds to androgen therapy (Chapter 54). The use of steroids in controlling hemolysis and severe thrombocytopenia is discussed in Chapter 54. Trials of intermittent as compared with continuous steroid therapy<sup>10</sup> have been too limited to evaluate with respect to antileukemic effect as compared with toxicity.

### Alkylating Agents

During early trials of *nitrogen mustard* it became evident that CLL patients treated with this drug were more likely to develop severe and even fatal (Table 49-2) pancytopenia than were patients with Hodgkin's disease given comparable doses. Consequently, the orally administered drugs have been preferred as their toxic manifestations usually can be controlled by discontinuing their use at the first evidence of marrow depression. *Chlorambucil*<sup>17,20,21</sup> is probably the drug most

used in the treatment of patients with CLL (Fig. 49-6). The results of a controlled trial suggested that it was slightly superior to busulfan in this disease.<sup>51</sup> *Cyclophosphamide* also has been used widely in treating patients with CLL and differs little, if at all, from chlorambucil in its effects.<sup>34</sup> Another alkylating agent, *elderfield pyrimidine mustard*, may be superior to either chlorambucil or cyclophosphamide for CLL therapy.<sup>37</sup> Chlorambucil is given in a daily dosage of 0.1 to 0.2 mg/kg or 0.4 mg/kg biweekly<sup>34a</sup>; cyclophosphamide, 1 to 2 mg/kg daily. The level of erythrocytes, platelets, and neutrophils must be carefully monitored and therapy should be discontinued with any evidence of its decline. Unfortunately, the numbers of these cells often are depressed for prolonged periods after stopping therapy and do not readily return to normal levels (Table 49-3). Somewhat better results than those cited in the table have been reported by others, at least during the first course of chemotherapy.<sup>20,24</sup> This is in contrast to other forms of leukemia; eg, anemia regularly improves with chemotherapy in patients with CML. The reason why normal blood cells respond

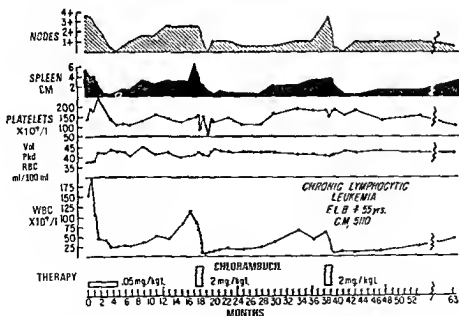


Fig. 49-6. Course of a patient with chronic lymphocytic leukemia who had marked leukocytosis but only slight anemia and thrombocytopenia and was treated at long intervals with chlorambucil

less consistently to alkylating agents given to patients with CLL than to patients with CML is not known.

It has been suggested, but in no sense proved, that administration of alkylating agents or irradiation therapy may precipitate autoimmune hemolytic anemia as well as other hyperimmune phenomena.<sup>36,69</sup> Most patients report symptomatic improvement during alkylating agent therapy and in most a decrease in the number of blood lymphocytes, usually accompanied by decrease in the size of lymph nodes and spleen, is observed (Table 49-3). The end point of a course of therapy is difficult to assign since depression of normal blood cells usually develops before blood lymphocytes return to normal or spleen and lymph node enlargement disappears. As to maintenance therapy with small doses of drug given daily, advocated by some, a controlled study showed no obvious advantage of this therapy over intermittent therapy.<sup>50</sup>

### Other Forms of Therapy

**Irradiation** of the spleen and other areas of enlarged lymphoid tissue,<sup>16,40</sup> extracorporeal irradiation of the blood,<sup>21,38,39</sup> irradiation of retroperitoneal nodes by introducing radioactive substances into lymphatics of the feet,<sup>15</sup> attempts to concentrate radioactive material in reticuloendothelial tissue by giving it in colloidal form,<sup>30</sup> spaced doses of whole-body irradiation using <sup>32</sup>P<sup>31,42,57</sup> or x-irradiation,<sup>33</sup> and a variety of drugs<sup>51,56</sup> will all reduce the total number of lymphocytes as will antilymphocyte serum (Chapter 55). Leukapheresis<sup>17</sup> and thoracic duct drainage<sup>9</sup> reduce blood lymphocyte levels and may induce a decrease in the size of lymph nodes and spleen.

**Complete remission**, as defined for acute leukemia with no residual evidence of disease, is most unusual in CLL.<sup>7,14,29</sup> In the only patient in whom we have observed a complete remission, the remission may have occurred spontaneously,<sup>14</sup> as it has in a few other patients.<sup>14,29</sup> The report<sup>62</sup> of improvement in CLL as polycythemia vera developed suggests that there are natural means of sup-

pressing the manifestations of CLL. Some authors have chosen to use a definition of remission that allows moderately severe thrombocytopenia and anemia to be present.<sup>33</sup> No form of therapy has been reported that leads to a rise in serum immunoglobulins, the factor that seems to be most responsible for infection—the major cause of death in CLL patients. The aberrant blastogenic response of blood lymphocytes cultured with phytohemagglutinin (PHA) changes toward normal when blood lymphocyte concentration has returned toward normal following therapy.<sup>8</sup> However, there is no evidence that serum gamma globulin levels and response to antigenic stimulation or frequency of infection are improved favorably by therapy.

Thus, it is difficult to be dogmatic concerning the treatment of patients with CLL. *Since evidence of benefit from therapy is equivocal, we prefer to allow many of our patients to remain untreated for prolonged periods, except for vigorous treatment when complications occur (Chapter 54).* When systemic chemotherapy is employed, a course of 40 mg, or less, of prednisone per day is given for four to six weeks and chlorambucil or cyclophosphamide is then added and the administration of prednisone is stopped. Alternatively, prednisone and an alkylating agent may be given in combination rather than sequentially. In a controlled clinical trial, chlorambucil, 6 mg/day, plus prednisone, 30 mg/day, for six weeks was found to be superior to chlorambucil alone with respect to tumor reduction and effect on levels of normal blood cells.<sup>29a</sup> The blood must be monitored, however, to make certain that more good than harm is being accomplished. As yet to be evaluated is intermittent therapy with cyclophosphamide and vincristine intravenously and prednisone orally (COP, Chapter 51). In general, in case of doubt, it is best to withhold therapy, thereby at least avoiding toxic injury induced by the therapeutic agent.

*To sum up*, the least important indication for therapy in CLL is the number of lymphocytes. Also of low importance are adenopathy and splenomegaly, unless these are very great and physically troublesome. Reas-

surance that these in themselves are not harmful or of serious prognostic significance often suffices. Of importance, on the other hand, are the development of anemia, thrombocytopenia (if severe), and impairment of immune defense mechanisms.

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## Hodgkin's Disease

- Classification
  - Histologic Classification
  - Clinical Staging
- Presenting Features
  - Symptoms
  - Physical Examination
  - Pattern of Spread
  - Hodgkin's Disease in Children
  - Laboratory Findings
- Staging Procedures
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- Therapy
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  - Palliative Radiotherapy
  - "Curative" Chemotherapy
  - Combination Chemotherapy-Radiotherapy
  - Palliative Chemotherapy

background. Three relatively recent developments are of particular significance: (1) realization that cure of the disease is a reasonable goal in many patients; (2) recognition of the prognostic implications of various histologic classes of HD; and (3) appreciation of the prognostic importance of the clinical extent of disease at the time of diagnosis.

### Classification of Hodgkin's Disease

At most treatment centers, two systems of classifying the disease are applied to each patient, one based upon the histologic appearance of the excised tumor (histologic classification) and the other determined by a thorough evaluation of the extent and location of tumor (clinical classification).

#### Histologic Classification

Hodgkin's disease is distinguished from other lymphomas by certain large, binucleate cells having vesicular nuclei and prominent eosinophilic nucleoli. These cells, called Reed-Sternberg cells, are scattered irregularly throughout Hodgkin's infiltrate. Although Reed-Sternberg cells<sup>103</sup> may be seen occasionally in hyperplastic or inflammatory lesions of lymph nodes, they remain the most reliable histologic marker of Hodgkin's disease, in the absence of which the diagnosis should not be made.

**H**ODGKIN's disease (HD) is a tumor of lymphoid tissue that is recognized by distinctive histologic changes in excised tissue. Although it can occur in persons of any age (Chapter 46), it is most commonly seen in young adults and is more common in males than in females (Chapter 46). The earliest descriptions of HD were discussed in Chapter 46 as were theories concerning the etiologic

Lymph nodes involved by Hodgkin's disease show either partial<sup>148</sup> or total obliteration of normal follicular and sinusoidal architecture by a diffuse and frequently mixed infiltration of lymphocytes, histiocytes, eosinophils, plasma cells, and neutrophils. Accompanying Reed-Sternberg cells may be either sparse or plentiful. Histologic subclassification of Hodgkin's disease relies principally on differences in the cellular composition of this infiltrate, particularly on variations in the proportions of lymphocytes and histiocytes that it contains. Both the Rye classification<sup>133</sup> (see below) and the prior Jackson and Parker<sup>70</sup> scheme acknowledge that when the nodal infiltrate is comprised predominantly of mature lymphocytes the

disease is usually localized at discovery and the prognosis is relatively favorable. In this *lymphocyte-predominant* form of Hodgkin's disease (Fig. 50-1) (Jackson and Parker: paragramuloma), Reed-Sternberg cells, eosinophils, and histiocytes are sparse so that the disease may be mistaken for diffuse lymphocytic lymphoma by the casual observer. Alternately one may find Hodgkin's infiltrate comprised principally of histiocytes and very few lymphocytes. Eosinophils and Reed-Sternberg cells occur in varying numbers in this *lymphocyte-depleted* form of Hodgkin's disease (Fig. 50-2) but are usually more common than in the lymphocyte-predominant form. A varying amount of nodal fibrosis is also seen, usually distributed in random or

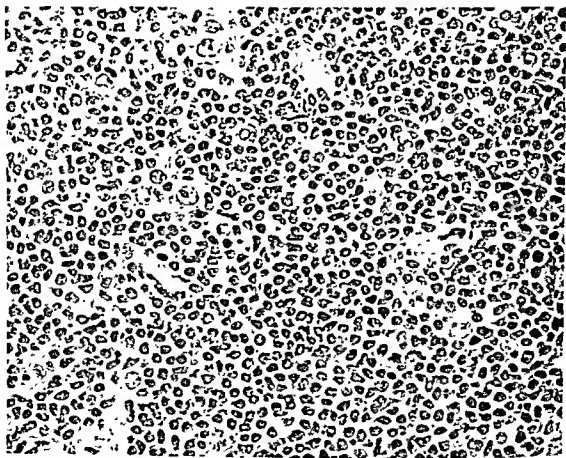


Fig. 50-1 Lymphocyte predominant Hodgkin's disease. The predominant cell is the small lymphocyte. Eosinophils and histiocytes are sparse and fibrosis is uncommon. Several Reed-Sternberg cells are seen in the upper left corner. Lymph node. H & E 200X. (Courtesy of Dr. R. W. McDivitt.)

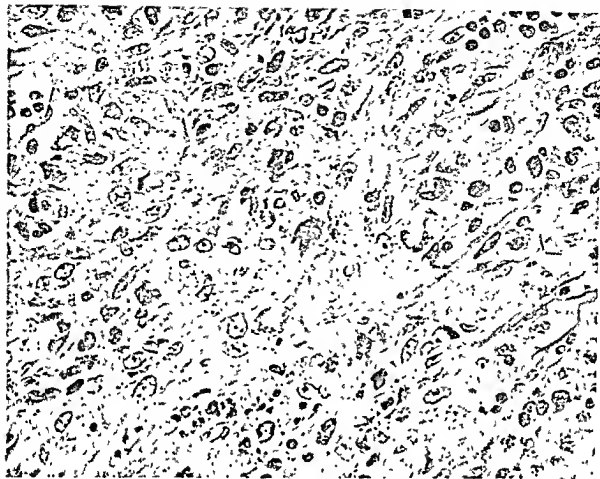


Fig 50.2 Lymphocyte-depleted Hodgkin's disease. The predominant cell is the histiocyte. Lymphocytes are sparse. A few scattered eosinophils are seen but usually fewer than in the mixed cellular and nodular sclerosing types. Lymph node. H & E 400X. (Courtesy of Dr R. W. McOwitt.)

linear fashion. Lymphocyte-depleted Hodgkin's disease (Jackson and Parker Hodgkin's sarcoma) is usually a disease of older individuals, is more frequently generalized (stage III or IV, Table 50-1) at discovery than is the lymphocyte-predominant form, and has the least favorable prognosis of all the histologic types. The lymphocyte-depleted and the lymphocyte-predominant variants of Hodgkin's disease are the least common of the four histologic types<sup>23</sup> (page 1539).

Most commonly, Hodgkin's infiltrate is of a mixed type and includes numerous eosinophils, neutrophils, plasma cells, and rather evenly balanced numbers of lymphocytes and histiocytes. Reed-Sternberg cells are usually plentiful. Jackson and Parker referred to this variety as Hodgkin's granu-

loma.<sup>70</sup> It comprised 80 to 90% of cases, and these varied considerably in clinical course and prognosis. Some criticism of the Jackson-Parker classification has been obviated by the more recent Rye classification,<sup>133</sup> which follows the suggestion of Lukes, Butler, and Hicks and divides the mixed group according to whether or not the infiltrate is nodular or diffuse in distribution.<sup>104,105</sup>

In *nodular sclerosing Hodgkin's disease* (Fig. 50-3), microscopic nodularity is produced by interlacing bands of collagen which divide the more cellular portions of the infiltrate into discrete islands. In addition to nodularity, this type of Hodgkin's disease is distinguished by Reed-Sternberg cells that differ somewhat from the ordinary variety. These cells, referred to as *lacunar Reed-Sternberg*

**Table 50-1. Clinical Staging Classification for Hodgkin's Disease (Rye Conference, 1965)\*†**

<i>Stage I</i>	Disease limited to one anatomic region or to two contiguous anatomic regions on the same side of the diaphragm
<i>Stage II</i>	Disease in more than two anatomic regions or in two noncontiguous regions on the same side of the diaphragm
<i>Stage III</i>	Disease on both sides of the diaphragm, but not extending beyond the involvement of lymph nodes spleen and/or Waldeyer's ring
<i>Stage IV</i>	Involvement of the bone marrow lung parenchyma pleura liver bone, skin, kidneys gastrointestinal tract, or any tissue or organ in addition to lymph nodes spleen, or Waldeyer's ring

All stages subclassified as 'A' or 'B' to indicate the absence or presence respectively of systemic symptoms. The following documented symptoms, otherwise unexplained are significant: (a) fever (b) night sweats and (c) pruritus. Hodgkin's disease limited to non lymphoid organs is excluded from the classification. Waldeyer's ring consists of tonsils adenoids and related lymphoid tissue of the oropharynx.

\*From Tubiana et al.<sup>155</sup> courtesy of the authors and Cancer Research.

†Now replaced by Ann Arbor Classification Table 50-2.

cells, have very faint cytoplasmic staining and are separated from adjacent cells by an empty space or lacuna, most probably as a result of fixational contraction. Nodular sclerosing Hodgkin's disease is most frequently a disease of young adults, usually localized (stage I or II) at discovery, favorable in prognosis, and most often found in the cervical regions and mediastinum.<sup>42</sup>

Hodgkin's disease of diffuse mixed pattern is referred to as *mixed cellular* Hodgkin's disease (Fig. 50-4). In this form, fibrosis is fairly common and focal necrosis is observed in as many as 25% of patients.<sup>158a</sup> Like the lymphocyte-depleted variety, it has a propensity to be generalized at discovery, and carries a less favorable overall prognosis than does either the nodular sclerosing or lymphocyte-predominant type. Nodular scler-

osing and mixed cellular Hodgkin's disease each account for about 40% of cases at diagnosis.<sup>22a</sup>

*One must not assume from the preceding outline that histologic classification of Hodgkin's disease is always precise.* Most series report somewhere between 5 and 10% of cases that seem to defy such classification; and variation occurs among pathologists in classifying the remainder.<sup>22a,42</sup> Certainly, the parameters defining the boundaries between mixed cellularity, lymphocyte-depleted, and lymphocyte-predominant types provide considerable latitude for variation, as do differences of opinion concerning whether or not disease of diffuse mixed cellularity, with lacunar cells but without obvious fibrosis or nodularity, should be included in the nodular sclerosing group.<sup>81,149</sup> Evidence also suggests that when repeated biopsies are obtained throughout the course of the disease, the histologic classification may not remain constant. Whereas nodular sclerosis seems most persistent, only about 10% of this type shifting to a different histologic type on repeated biopsy, approximately 60% of other types may be expected to show variability in histologic classification if more than one biopsy specimen is obtained.<sup>249</sup> These factors must be considered in evaluating the overall significance of histologic classification.

Thus the classification most generally adopted at the present time<sup>131</sup> recognizes a rather distinctive form of Hodgkin's disease, nodular sclerosis, and a continuum of pathologic appearance that can be divided into lymphocyte-predominant, mixed cellularity, and lymphocyte-depleted stages (Fig. 50-5). It has been suggested that a cellular phase may precede the formation of collagen bands.<sup>81</sup> Nodes with the lacunar type of Reed-Sternberg cell may represent an early stage of nodular sclerosis.<sup>131,149</sup> In nodular sclerosing Hodgkin's disease, areas of necrosis may be observed, as may large, foamy macrophages.<sup>158a</sup> It should also be noted that although the Reed-Sternberg cell is so characteristic of Hodgkin's disease, it probably is not a neoplastic cell itself, since DNA synthesis is not observed in such cells.<sup>129</sup>



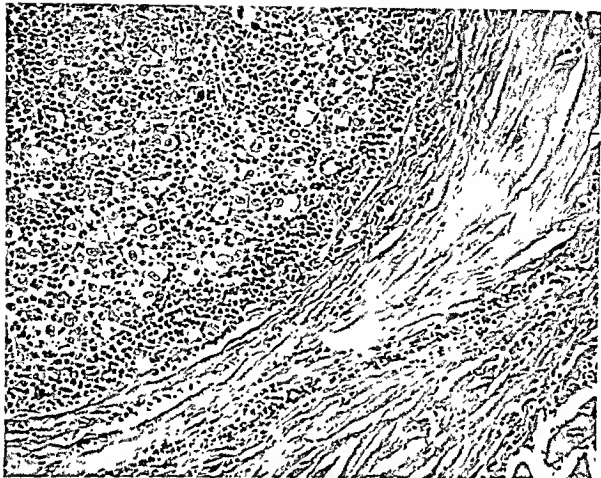


Fig 50-3. Nodular sclerosing Hodgkin's disease. Broad bands of collagen separate cellular nodules of tumor. Numerous lacunar Reed-Sternberg cells are intermixed with lymphocytes, histiocytes, plasma cells, and eosinophils. Lymph node. H & E 160X. (Courtesy of Dr R. W. McDivitt.)

**FREQUENCY OF VARIOUS TYPES.** Since diagnostic criteria are not exact, some variation in the frequency of various types as classified by different pathologists is to be anticipated. In one series of 302 patients, 31% of the cases were classed as nodular sclerosis, 27% as lymphocytic predominance, 32% as mixed cellularity, and 10% as lymphocyte depleted.<sup>154</sup> In another series of 252 patients the percentages were 45, 14, 21, and 19, respectively.<sup>112</sup> In still another referral center, nodular sclerosis was considered to be present in the majority of patients.<sup>81,86</sup> Some of the variation in percentage of histologic type from one to another series may be explained by different criteria for selecting patients for admission to an institution. However, as mentioned above, there is a considerable de-

gree of disagreement as to histologic type between different pathologists.<sup>33</sup>

The prognostic significance of the histopathologic classification and its relation to clinical staging are discussed on page 1553.

### Clinical Staging

Beginning with Trousseau in 1865, clinicians have proposed various clinical classifications of HD.<sup>122</sup> In order to provide some uniformity in reporting, a group of investigators agreed to utilize a single classification on a trial basis at the Rye (New York) Conference<sup>155</sup> (Table 50-1). In this classification, stages I through III represented increasing numbers of involved lymph node groups and IV represented extranodal spread of disease.

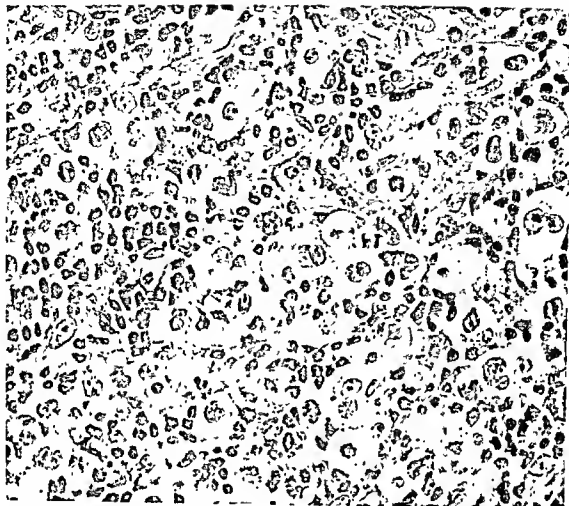


Fig 50-4 Mixed cellular Hodgkin's disease containing numerous lacunar histiocytes. Some observers include tumors of this histology in the nodular sclerosing category, even though neither nodularity nor sclerosis is obvious. Lymph node. H & E 400X. (Courtesy of Dr. R. W. McDivitt.)

Addition of A or B to each respective Roman numeral indicated absence (A) or presence (B) of fever, night sweats, or pruritus.

The classification of HD has continued to change as knowledge concerning which lesions are and which are not amenable to cure by radiotherapy has evolved. The most recently agreed upon classification<sup>26</sup> (Table 50-2) incorporates an important observation made since the Rye classification was recommended in 1965; namely, if extra-lymphatic disease is localized and related to adjacent lymph node disease it has approximately the same prognosis as that of disease which has not spread beyond that node. The disease-free

interval after therapy for stages II and III with or without local extension to adjacent non-lymphatic tissue has been found to be about the same.<sup>112</sup> Consequently, localized parenchymal lung involvement adjacent to an involved hilar node is no longer considered stage IV disease. Such local extension to pleura, bone, skin, or other soft parts also may be observed.<sup>24</sup> Procedures used to establish the stage should be incorporated into the staging classification (Table 50-3). Other changes from the Rye classification include a change in stage I-II designations and a change in symptoms that are considered to be of prognostic significance. Disease limited

to two contiguous node groups is now stage II rather than stage I, since survival with two groups on one side of the diaphragm more closely approximates survival when three groups are present than when only one is found.<sup>156</sup> Pruritus has been dropped as a significant symptom and weight loss has been added, to agree with recent findings regarding the significance of these manifestations.<sup>156</sup>

Examples of how such a classification is applied are given in Table 50-3. Additional changes in staging classification may be anticipated as knowledge of response to therapy as related to other abnormalities, such as degree of immunologic impairment, is acquired. Furthermore, the exact location of nodes may prove important in staging. Among 101 patients having high cervical nodes (submental, submaxillary, and jugular) as the presenting manifestation, involved supraclavicular and axillary nodes frequently were present, but in all these patients the medi-

astinum was spared.<sup>154</sup> In contrast, when the primary site (as defined by largest node size) appeared to be the lower cervical or supraclavicular region, the majority of the patients gave evidence of mediastinal disease.

## Presenting Features

### Symptoms

An enlarged painless cervical node (or nodes) without systemic symptoms is the most common presenting manifestation.<sup>70</sup> A history of fluctuation in size of the enlarged nodes is obtained frequently, as is spontaneous remission and exacerbation of other symptoms.<sup>78</sup> Fever, without other symptoms of infection, is relatively common, as are drenching night sweats, but chills are uncommon. *Periodic, regularly recurring* bouts of fever as described by Murchison,<sup>111</sup> Pel,<sup>121</sup> and Ebstein<sup>11</sup> may be experienced occasionally. However, virtually any type of fever

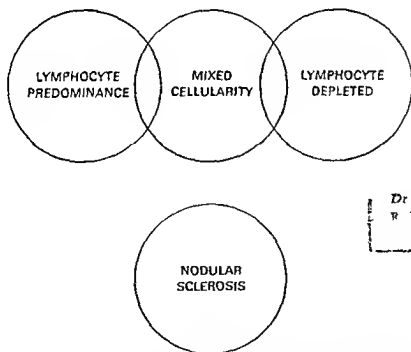


Fig. 50-5 Histologic classification of Hodgkin's disease (Rye conference<sup>123</sup> modification of Lukes and Butler's classification).<sup>101</sup> Lymphocyte predominance, mixed cellularity, and lymphocyte-depleted varieties represent a continuum of disease (indicated by the overlapping circles), while nodular sclerosis is a distinct variety of disease (See text for histologic descriptions.)

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**Table 50-2. Current Recommended Clinical Staging for Hodgkin's Disease ("Ann Arbor" Classification)\***

<b>Stage I</b>	Involvement of a single lymph node region or of a single extralymphatic organ or site
<b>Stage II</b>	Involvement of two or more lymph node regions on the same side of the diaphragm or localized involvement of an extra-lymphatic organ or site and of one or more lymph node regions on the same side of the diaphragm. Optionally the number of node regions involved is indicated by a subscript
<b>Stage III</b>	Involvement of lymph node regions on both sides of the diaphragm. This may or may not be accompanied by localized involvement of an extra lymphatic organ or site or involvement of the spleen or both
<b>Stage IV</b>	Diffuse or disseminated involvement of one or more extra lymphatic organs or tissues with or without associated lymph node enlargement. The reason for classifying a patient as stage IV is indicated by defining the site or sites by symbols. Extra lymphatic organs are defined as those other than lymph nodes, spleen, thymus, Waldeyer's ring, appendix, and Peyer's patches. Liver or bone marrow involvement always indicates Stage IV disease

**A-B Subclassification.** A refers to patients without B with defined general symptoms as follows:

Unexplained fever with oral temperatures above 38° C

Night sweats

Unexplained weight loss of more than 10% of body weight in the six months before admission

Unexplained pruritus no longer justifies B classification nor do other symptoms such as alcohol-induced pain

It is suggested that each patient be given a clinical designation based on the history, physical and radiologic examinations including isotopic scans and liver function tests as well as a histologic designation based on all biopsy material obtained subsequent to the initial diagnostic biopsy (Table 50-3)

\*From Carbone et al.<sup>74</sup> courtesy of the authors and Cancer Research

**Table 50-3. Examples of Staging Classification of Hodgkin's Disease**

**CS I-A PS I<sub>S-H-N-</sub>**

**Interpretation:** CS I-A (clinical stage I-A), PS I (pathologic stage<sup>75</sup> I), S— (splenectomy done and negative), H— (negative biopsy of liver), N— (additional lymph nodes negative), and M— (marrow biopsy negative).

**CS II-A PS III<sub>S-H-N-M-</sub>**

**Interpretation:** Clinical stage II-A but Hodgkin's involving spleen and abdominal nodes found at laparotomy, therefore, pathologic stage III-A, liver and marrow biopsies negative

**CS III-B PS IV<sub>H+</sub>**

**Interpretation:** Clinical stage III-B. Pathologic staging procedures terminated when liver biopsy was positive

\*Based on histologic examination of tissues removed at laparotomy

pattern from continuous low-grade to hectic daily swings may be observed.<sup>14,100</sup> Fever due to infection is uncommon until terminal stages of the disease are reached.<sup>14,100</sup> Weight loss may occur. Generalized pruritus without a rash is sometimes a very troublesome symptom. A peculiar and unexplained symptom is the development of pain very shortly after drinking small amounts of alcohol.<sup>31,73</sup> This type of pain may be elicited in as many as 17% of patients if alcohol tolerance tests are done,<sup>127</sup> but is less common as a spontaneous complaint.<sup>86</sup> The pain usually is at a site of HD infiltration and is transient, but may be severe. This symptom is reported in association with other conditions only rarely, but has been described by patients with eosinophilic granuloma of bone,<sup>99</sup> osteomyelitis, fractures, or carcinoma.<sup>73</sup> Other types of pain are infrequent unless bone is invaded or nodes compress nerves or nerve roots; the compression of nerves or nerve roots most commonly occurs in the retroperitoneal space. Complaints relating to extranodal disease such as cough from pulmonary infiltration, jaundice from hepatic invasion, or ab-

**Table 50-4. Frequency of Involvement of Various Lymphoid Structures in 340 Patients with Hodgkin's Disease\***

Site	Frequency	Percentage of Patients with Specified Area as Only Involved Site
Left cervical and/or supraclavicular nodes	71%	43%
Right cervical and/or supraclavicular nodes	59%	22%
Mediastinal nodes	62%	9%
Hilar nodes	11%	0
Left axillary nodes	26%	5%
Right axillary nodes	23%	9%
Para-aortic nodes	34%	2%
Iliac, inguinal, and/or femoral nodes	16%	9%
Spleen	13%	0

\*Adapted from Kaplan.<sup>85</sup>

dominal pain from disease of bowel or of thyroid enlargement are unusual, particularly as the only complaint. Initial symptoms of disease apparently limited to extranodal tissue<sup>89,137,146</sup> are much rarer in HD than in the non-Hodgkin lymphomas (Chapter 51).

### Physical Examination

Enlarged cervical or supraclavicular lymph nodes, especially those of the left side, are the most common physical abnormalities (Tables 50-4 and 50-5) (Fig. 50-6). Mediastinal disease (Fig. 50-7) is next most common, but usually is associated with supraclavicular disease; it is not a common site of localized disease (Table 50-4) nor is it often the largest area of involvement (Table 50-5). The majority of patients with supraclavicular-mediastinal disease have the nodular sclerosing variety of HD.<sup>85</sup> Enlargement of axillary or inguinal nodes without cervical adenopathy is less common than the converse. Generalized lymphadenopathy is present in a minority of patients. The nodes usually are not tender and changes in the overlying skin are unusual. When tenderness and skin changes are present they are thought to reflect rapid growth with stretching of nodal capsules. The nodes feel firm, but are not hard and are somewhat "rubbery," but, when extensive sclerosis or fibrosis is present, as in some patients with the nodular sclerosing or

lymphocyte depleted varieties, the nodes may be quite hard. In most patients the nodes are discrete and freely movable. Calcification may occur in nodes after they have been irradiated.<sup>167</sup>

*Splenomegaly* is present in a minority of patients and *hepatomegaly* is even less common. Palpable enlargement of the spleen has proved to be due to HD involvement of the spleen in only a third to one half the subjects.<sup>40,86</sup> Hodgkin's disease limited to the spleen is quite rare,<sup>85,154</sup> but we have seen such a patient; this patient had severe fever and weight loss at the time of death and the only disease found at autopsy was HD diffusely involving an enlarged spleen.

As discussed in Chapter 54, virtually any organ can be invaded by HD. However, evidence for disease in areas other than lymph

**Table 50-5. Apparent Site of Onset of Hodgkin's Disease as Judged by the Largest Area of Involvement at the Time of Diagnosis (348 Patients)\***

High cervical nodes	29%
Supraclavicular nodes	41%
Mediastinal structures	11%
Axillary nodes	4%
Abdominal nodes	13%
Spleen	1%

\*Adapted from data of Teillet et al.<sup>154</sup>

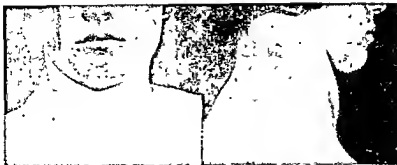


Fig 50-6 Enlargement of glands in the posterior triangle of the right side of the neck in a patient with Hodgkin's disease

nodes and spleen is present only in a small minority of patients at the time of diagnosis (page 1552). Abnormal physical or roentgenographic findings at diagnosis disclosed HD of lung in 11%, of the liver in 6%, of bone in 6%, and of pleura, skin, or other soft tissue in 2% or fewer in one series of patients.<sup>112</sup> As the disease advances the lung and liver become involved in many patients as do kidneys, stomach, and bowel in a lesser number (see Chapter 54).

#### Pattern of Spread

The apparent pattern of spread of HD is consistent with a unicentric origin in most patients, although multicentric origin in some patients cannot be ruled out.<sup>80</sup> When first consulting a physician, approximately 90% of the patients have disease in which contiguous spread via lymphatic channels could have occurred, at least if retrograde thoracic duct extension from supraclavicular to para-aortic nodes takes place. Retrograde lymphatic spread from involved nodes is thought to be the mechanism of spread to the skin.<sup>36</sup> Splenic disease may represent spread from para-aortic nodes, and hepatic disease probably results from hematogenous spread from the spleen.<sup>81</sup> Alternatively, spread to the spleen may be hematogenous and involved abdominal nodes may represent spread from the spleen.<sup>5</sup> Parenchymal lung involvement appears to be the consequence of direct invasion from involved hilar nodes or mediastinum.

Hematogenous spread to marrow or liver may be predicted to some degree if vascular invasion by HD can be demonstrated in nodes and spleen.<sup>130</sup> However, vascular invasion is not necessary for hematogenous spread to occur. The demonstration of Reed-Sternberg cells in thoracic duct drainage in all of seven patients found to have high para-aortic node involvement<sup>46</sup> suggests that cells from nodes involved with HD are regularly delivered to the blood of some patients. There is some evidence that the pattern of spread differs according to the histologic type of HD.<sup>67</sup>

#### Hodgkin's Disease In Children

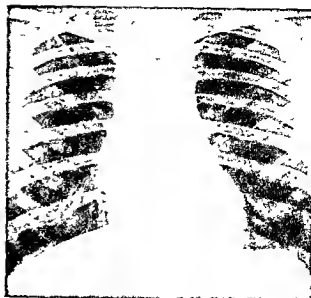
HD in children is somewhat uncommon, but is quite similar to the disease observed in adults.<sup>47,74,125,130a,147</sup> The course in children may be more rapid than in adults if extensive disease is present at the time of diagnosis,<sup>47</sup> but, in general, response to therapy and survival are similar to those expected in adults.<sup>47,74,125,140a,171</sup> HD is exceedingly uncommon in children younger than age three, but has been reported in an infant of five months who also had thymic aplasia.<sup>160</sup> Strum and Rappaport<sup>147</sup> reviewed five other reports of HD in infancy and found none of them convincing. There is a marked male predominance in children and the pathologic classification is usually either nodular sclerosis or lymphocyte predominance.<sup>147</sup>

### Laboratory Findings

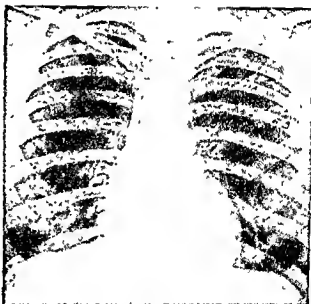
Anemia is present in no more than 50% of the patients at the time of diagnosis (Table 50-6).<sup>97</sup> Coombs' positive hemolytic anemia can be found when the diagnosis is made,<sup>16</sup> but is more common during the terminal stages of the disease<sup>45</sup> (Chapter 54). In most patients the anemia is mild, normochromic, and normocytic and is accompanied by a normal or reduced percentage of reticulocytes. Hypoferremia may be present, but, unlike anemias associated with many forms of chronic disease (Chapter 18), this is not necessarily accompanied by decreased transferrin levels.<sup>72</sup> Erythrocytosis was reported in one patient with HD.<sup>20</sup>

The leukocyte count may be increased<sup>97</sup>; leukopenia is unusual (Table 50-6). Leukocytosis most often reflects neutrophilia. Eosinophilia is more frequently written about than observed. However, extreme degrees of eosinophilia may be present.<sup>8,107,141</sup> Modest elevation of the leukocyte alkaline phosphatase usually is found during periods of active disease. Monocytosis may accompany the neutrophilia<sup>22</sup> and an increase in basophilic leukocytes is observed occasionally. Lymphopenia is present in one third<sup>36</sup> or more of HD patients (Table 50-6) and has long been recognized as a frequent finding.<sup>22</sup> The percentages of large and medium-sized lymphocytes and of lymphocytes synthesizing DNA are increased during active phases of HD and plasma cells may be observed in the blood.<sup>36</sup> Lymphocytes with nucleoli are found with greater than normal frequency.<sup>127</sup> The output of small lymphocytes from the thoracic duct decreases in lymphopenic patients.<sup>46</sup> Abnormal-appearing cells may be found on blood smears or if leukocytes are concentrated.<sup>15,58</sup> Some of these are quite nonspecific and may be found in patients with viral diseases or even in normal persons.<sup>58</sup> In that category are abnormally large monocytes with large vacuoles and large lymphocytes with deeply basophilic cytoplasm.

Reed-Sternberg cells may be observed in the blood; when leukocyte concentrates were examined they were found in 12%<sup>58</sup> to 19%<sup>15</sup>



A



B

Fig. 50-7. A, Enlargement of the glands in the upper mediastinum on the right and in the right supraclavicular region, in a patient with Hodgkin's disease. B, Normal mediastinal shadow in the same patient 2½ years following roentgen irradiation

of HD patients. Rarely, with widespread disease, they are numerous, so much so that such cases might be termed Reed-Sternberg cell leukemia<sup>137</sup>; in one patient in excess of  $100 \times 10^9$  Reed-Sternberg cells/l of blood were present.<sup>144</sup> Another abnormal-appearing

Table 50-6. Blood Values in Hodgkin's Disease at the Time of Diagnosis\*

Clinical Stage	Number of Patients	Hemoglobin	Total Leukocytes		Eosinophils	Monocytes	Lymphocytes
		Less than 13 g/dl (%)	More than $10 \times 10^9/l$ (%)	Less than $5 \times 10^9/l$ (%)	More than $0.7 \times 10^9/l$ (%)	More than $0.8 \times 10^9/l$ (%)	Less than $1.5 \times 10^9/l$ (%)
I	17	24	0	0	0	6	6
II A	34	29	26	3	6	6	50
II-B	21	57	43	0	10	14	67
III A	12	42	17	17	8	0	42
III B	11	55	45	9	9	18	45
IV	7	71	57	0	29	14	43
All stages	102	41	28	4	8	9	44

\*Based on values published by Kaplan <sup>24</sup>

cell, possibly a precursor of the Reed-Sternberg cell, is even more often present in the blood.<sup>58</sup> This cell, as large as 40  $\mu$ m in diameter, has a nucleus that is oval or lobulated with fine, reticular chromatin and one or more large nucleoli and moderately basophilic cytoplasm. The presence of this cell or of Reed-Sternberg cells in the blood has been considered to be indicative of HD in the spleen.<sup>58</sup>

Although anemia, neutrophilia, and lymphopenia are less common with stage I disease than with other stages, they do not necessarily reflect the extent of disease (Table 50-6).

*Platelet counts* usually give normal values<sup>97</sup> although the count may be above normal and large bizarre platelets may be seen occasionally.<sup>22</sup> The finding of thrombocytopenia suggests bone marrow involvement. Pancytopenia is rare and usually reflects marrow involvement.

The *bone marrow* often is normal when examined by aspiration and biopsy. An increased myeloid:erythroid ratio may be observed, reflecting increased neutrophil production,<sup>7</sup> decreased erythrocyte production, or both. Marrow biopsy discloses HD (Plate III) in approximately 5% of patients at the time that the diagnosis is made.<sup>135</sup> HD in marrow may not be predictable from changes in the blood; in one study three of 11 patients

with marrow involvement were not anemic.<sup>50</sup> In other patients, some degree of fibrosis may be observed or nonspecific granulomas may be found.<sup>61,131,135</sup> The pathologic significance of these findings and the frequency with which HD in bone marrow is missed because of random sampling are questions that must await long-term follow-up of large numbers of such patients.<sup>131,135</sup> Reed-Sternberg cells may be recognized in smears made from aspirates,<sup>61,168</sup> but biopsy study is preferable for their demonstration.<sup>61,131</sup> Hodgkin's disease on rare occasions may appear to be limited to the bone marrow even after careful autopsy examination.<sup>163</sup> One of our patients, in whom this diagnosis was made ante mortem, complained of radiating abdominal pain, increasing weakness, and loss of weight and was found to have fever of the Pel-Ebstein type. There was marked pallor and slight hepatic enlargement as well as abdominal distention, but there was no adenopathy or splenomegaly and no masses or glands could be demonstrated in the abdomen; however, bone lesions were present (Fig. 50-8). The blood counts suggested aplastic anemia (Hb, 5 g/dl; WBC,  $1.2 \times 10^9/l$ ; platelets,  $88 \times 10^9/l$ ), but a moderate increase in percentage of reticulocytes (4.9%), together with the presence of nucleated red cells and occasional polychromatophilic corpuscles in the blood smears,





Fig 50-8. Two small areas of decreased density in the bones of the skull resembling metastases due to carcinoma, in a patient with 'bone marrow' Hodgkin's disease. The patient had severe anemia, leukopenia, and thrombocytopenia without glandular enlargement or splenomegaly. The only other bone lesion was increased osteoporosis of the bones in the region of the left wrist.

indicated the myelophthisic character of the anemia.

*Sedimentation rate and serum protein electrophoresis* often are abnormal and, while not of great diagnostic aid, may prove useful in judging whether disease activity is or is not present.<sup>13</sup> Albumin may be decreased and gamma globulin levels are often elevated in a diffuse pattern. Alpha-2 globulin levels often are quite high and may be so peaked as to mimic a para-protein spike (Fig. 50-9). Alpha-1 globulin usually increases in the presence of fever. Other tests that are likely to give abnormal results in patients with active disease and that to some extent tend to mirror the degree of disease activity are C-reactive protein,<sup>165</sup> hydroxyproline levels in plasma,<sup>95</sup> serum complement, serum copper and zinc, leukocyte alkaline phosphatase, haptoglobin, serum protein-bound hexose, and hexosamine and bradykinin tests.<sup>86</sup> The serum copper level appears to be a very sensitive index of the presence of disease.<sup>154a</sup> However, there is little evidence that any of these tests are more specific than the erythrocyte sedimentation test (ESR). On the other hand, if tumor-associated antigens are

shown to be present in most patients with active disease,<sup>30a</sup> this test should become the best means of determining whether or not there is active disease.

*Uric acid* excretion may be increased, but hyperuricemia is uncommon except during rapid tissue destruction by therapy. Even then, hyperuricemia of a degree that would

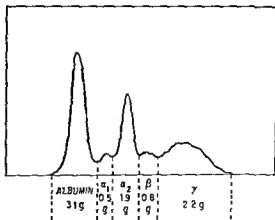


Fig. 50-9. Serum protein electrophoretic pattern from a patient with untreated, stage II-B Hodgkin's disease. Immunoelectrophoresis indicated that the alpha 2 elevation was not due to a paraprotein; note the diffuse elevation of gamma globulin.

produce renal impairment (Chapter 54) is unusual. Consequently, therapy for hyperuricemia rarely is indicated. *Hypercalcemia* is uncommon and usually reflects disease of bone (Chapter 54) although roentgenographic evidence of bone disease may be absent.<sup>79</sup> Serum *alkaline phosphatase* may be increased as a result of liver disease and less frequently because of bone disease.<sup>4</sup> This increase, even when identified as the liver isozyme, is not diagnostic of hepatic parenchymal HD, but when such disease is present the value almost always is elevated.<sup>4</sup>

**IMMUNE SYSTEM.** *Hypogammaglobulinemia* and reduced ability to form circulating antibodies are unusual<sup>12,13</sup> and when they are found, widespread severe disease almost always is present. We have encountered these changes at the time of diagnosis only in one patient (Fig. 50-15).

**Anergy** as reflected by loss of previously existing skin hypersensitivity to agents such as old tuberculin, mumps, trichophyton, histoplasma, and varidase, or inability to acquire such sensitivity as tested with dinitrochlorobenzene is a common finding.<sup>2,139</sup> (Chapter 46) In the presence of anergy, lymphocyte conversion with phytohemagglutinin<sup>60,119</sup> often is abnormal. The presence of anergy interrelates to some degree with the clinical stage of disease, the pathologic classification, and the level of circulating lymphocytes.<sup>2,60</sup> Anergy is more often associated with stage III or IV disease than with stage I or II. It is more commonly found in the lymphocyte-depleted variety of HD than when there is lymphocyte predominance and is more likely to be found when there is lymphocytopenia than when blood lymphocytes are present in normal concentrations.<sup>2</sup> Whether anergy is present because of extensive disease or whether extensive spread of HD has occurred because of anergy are important, but unresolved, questions. Deficiency of pyridoxal phosphate often is demonstrable in patients with active HD and has been suggested as a possible cause of anergy.<sup>28</sup>

When a therapeutically induced remission has continued for some time the above meas-

ures of cellular immunity often return to normal.<sup>28,60</sup>

## Staging Procedures

The therapeutic approach to the patient with HD (page 1556) is governed by the extent of disease at the time of diagnosis. Recognition of the fact that most patients with sharply localized HD can be cured by radiotherapy has led to continuing refinement of means for determining the extent of disease. However, the number of staging procedures that are carried out should be geared to the intended means of treatment. For instance, if it is the practice of the physician to give "total nodal" irradiation (page 1557) to all patients except those with stage IV disease, then only those procedures designed to detect stage IV disease should be employed. Conversely, if more limited radiotherapy is planned for limited disease, more extensive staging is required.

Once a pathologic diagnosis of HD has been established, additional studies in logical sequence are indicated. Detailed guidelines have been published.<sup>136</sup> The history should be reviewed with particular attention to the presence or absence and severity and duration of fever, night sweats, and weight loss as well as pruritus, alcohol-induced pain, and whether or not hydantoin-type drugs have been taken by the patient, since these may be associated with the development of lymphadenopathy (page 1553). All commonly involved lymph node areas should be carefully palpated and uncommonly involved sites such as epitrochlear, parascapular, and popliteal areas should also be examined. The size of the spleen and of the liver should be determined, the skin should be examined for suspicious lesions, and the skeleton should be palpated in search of areas of bone tenderness.

An x ray of the chest should be obtained and, if any suspicious areas are present, tomograms should be done. A bone marrow biopsy should be carried out. The marrow is involved in few patients with HD (page 1546), but, since the procedure is simple and since

positive results indicate stage IV disease, this procedure is advisable early in the work-up.

Liver function tests should be performed; the serum alkaline phosphatase level, especially, should be determined. Certain liver function tests, such as the BSP, have no apparent value in predicting the presence or absence of HD of the liver.<sup>40</sup> If the liver is enlarged or if it is of normal size but the function tests give markedly abnormal findings, isotopic scan of the liver and spleen may be advisable.<sup>108a</sup> If these studies suggest involvement of the liver, needle biopsy may be helpful. Multiple biopsies during abdominal exploration more often give positive findings than does single closed needle biopsy, but if the latter yields positive indications of HD, laparotomy becomes unnecessary as this alone justifies stage IV classification. An alternative to closed needle biopsy or exploratory laparotomy is peritoneoscopy, thereby obtaining a liver biopsy specimen under direct vision. In 38 selected patients, six were found to have HD of the liver by this procedure, a figure comparable to that obtained in similar patients subjected to laparotomy.<sup>41</sup> Of 21 of those patients in whom closed liver biopsies showed no abnormality, positive findings were obtained on peritoneoscopy in two.

Roentgenograms of vertebrae, pelvic, and proximal long bones as well as special films of any area of bony tenderness should be obtained. The laboratory procedures described above, in addition to examination of the blood for anemia, assessment of leukocytes and platelets, determination of ESR, blood urea nitrogen, serum uric acid, and blood glucose, and serum protein electrophoresis, as well as urinalysis, should be carried out in all patients, irrespective of their clinical staging classification as judged by physical examination. Additional studies will depend upon the extent of disease apparent at this point in the work-up and upon therapeutic intentions.

If "total nodal" irradiation (page 1557) is to be used for stages I, II, and III, then the only real consideration is to rule out the

possibility of stage IV. If the bone marrow, skeletal, lung, liver (including biopsy), and skin examinations do not show involvement, then the evaluation has been completed. If radiotherapy is to be limited to one side of the diaphragm when HD appears to be so limited and/or if chemotherapy is to be used for stage III-B, then further evaluation is in order. The common situation is clear evidence of disease above the diaphragm, with no or questionable evidence of subdiaphragmatic disease (clinical stage I, II-A, or II-B). In this circumstance, questionably enlarged inguinal nodes can be studied by excisional biopsy. A bilateral, lower-extremity lymphangiogram also can be obtained.

*Lymphography*<sup>32</sup> is performed by identifying a lymphatic vessel through a small incision on the upper surface of each foot and injecting radiopaque dye into the vessel. The dye can be observed in inguinal, iliac, and para-aortic nodes within 24 hours. Nodes involved with HD often are larger than normal and have rounded, punched-out appearing filling defects giving them a "lacunar" or "foamy" pattern.<sup>159</sup> However, this appearance is also seen in other types of lymphoma, fungal diseases, and sarcoidosis, to name but a few conditions; consequently, it is in no sense diagnostic of HD.<sup>159</sup> It is noteworthy that pulmonary symptoms may be produced when the dye enters the pulmonary vasculature; the test is therefore contraindicated in patients with any significant form of pulmonary disease.

If multiple, enlarged retroperitoneal nodes with the characteristic foamy "lymphomatous" appearance are present, one may choose to make a presumptive diagnosis of stage III disease without histologic proof, particularly when the patient is symptomatic (III-B). However, since there is a possibility that nodes considered positive on lymphangiography may not reveal HD on pathologic study (page 1550),<sup>159</sup> an exploratory laparotomy may be advisable. Inferior vena-cavography is of some supplemental help in detecting retroperitoneal disease, but intravenous pyelography rarely gives added information.<sup>93</sup>

If the lymphangiogram gives negative or equivocal findings, exploratory laparotomy<sup>118a</sup> should be considered. At the time of laparotomy, all accessible abdominal node areas are observed and/or palpated, the spleen is removed, and a wedge biopsy and multiple needle biopsies of the liver are carried out. Lymphangiography may reveal enlarged nodes in the upper retroperitoneal chain, which is not easily accessible to the surgeon and may guide him to suspicious nodes. It is therefore advisable to undertake this procedure routinely before performing an abdominal exploration. It may be advisable to obtain a large section of marrow from the iliac crest during the exploratory procedure.<sup>13a</sup> At the same time, in the female the ovaries and fallopian tubes can be moved to a more lateral position so that the ovaries are spared direct irradiation when radiotherapy employing the "inverted Y" is to be given subsequently<sup>10</sup> (page 1557).

In summary, *laparotomy and splenectomy* may be considered if: (1) stage IV disease is not certain; or (2) the patient appears to be in stage I or II and radiotherapy will be limited to the "mantle" (page 1557) if the abdomen proves to be free of HD; or (3) the patient appears to be in stage II-B and chemotherapy will be given if the abdomen contains HD.

Other studies that may be of some aid include whole body gallium<sup>124,127</sup> and selenium<sup>65</sup> scintigrams and studies of delayed hypersensitivity.

#### Value of Lymphangiogram, Exploratory Laparotomy and Splenectomy

A lymphangiogram was reported to be abnormal in 0 to 36% of patients thought to have stage I disease and in 14 to 51% of patients thought to have stage II disease in whom no palpable disease was found below the diaphragm.<sup>18</sup> In patients with positive lymphangiographic findings, pathologically positive nodes have been demonstrated at laparotomy in approximately 70 to 90%<sup>40,86,128</sup> (Table 50-7). Considering the fact that positive nodes are undoubtedly missed in certain instances, this indicates an accuracy in excess of 80% for positive lymphography.<sup>134</sup> When the lymphangiogram gives negative or equivocal evidence, positive nodes have been found in 10 to 30% of patients subjected to laparotomy.<sup>128,134</sup> In most instances, positive nodes found at laparotomy in patients with negative lymphangiographic findings have been in areas not filled by bipedal lymphangiography. Usually these will be nodes in the hilar area of the spleen (Table 50-7), but less commonly nodes of the mesen-

Table 50-7. Results of Laparotomy in 100 Consecutive Untreated Patients with Hodgkin's Disease Examined at Stanford University\*

Site	Preoperative Assessment	Number of Patients	Percent of Patients in Whom Hodgkin's Disease Was Found at the Site	
			Para-aortic	Splenic Hilar
Abdominal nodes	Positive lymphangiogram	23	74	4
	Negative lymphangiogram	57	4	9
	Equivocal lymphangiogram	20	15	5
Spleen	Splenomegaly present	16		50
	Splenomegaly absent	84		24
Liver	Liver disease suspected	15		7
	Liver disease not suspected	85		2

\*Adapted from Table 4.6 of Kaplan.<sup>14</sup>

tery, celiac axis, or porta hepatis region will be infiltrated with HD. The last areas are involved only rarely; Kaplan<sup>86</sup> reported that only two of 340 patients had HD in mesenteric nodes. Thus, technically good lymphangiography gives reasonably accurate information, perhaps more accurate than surgical exploration of the retroperitoneal node chain. Small series suggesting a high degree of inaccuracy for lymphangiography<sup>18,158</sup> have been contradicted by subsequently published series which included much larger numbers of patients.<sup>63,86,134</sup> Nevertheless, every pattern of lymphoma may be simulated by a reactive process.<sup>22a,119a</sup>

Whether or not there is *splenic disease* is perhaps the most useful information obtained at laparotomy. This is determined by splenectomy and multiple-section examination. A palpably enlarged spleen is not necessarily enlarged because of HD, as mentioned earlier (page 1543). Conversely, HD may be found in grossly normal-appearing spleens in almost one fourth of the patients<sup>131</sup> (Table 50-7). If para-aortic nodes are involved, splenic involvement is likely. Of 21 patients with para-aortic lymph node invasion, 15 were found to have splenic involvement.<sup>128</sup> Liver involvement is unusual in the absence of

splenic disease.<sup>134</sup> In as many as 20% of patients with involved para-aortic lymph nodes or spleen, hepatic invasion was demonstrated.<sup>40</sup>

### Frequency of Various Clinical Stages

Careful staging reveals previously unsuspected areas of disease in a significant number of patients. Even so, it would appear that at least 50% of patients when first seen have stage I or II disease and fewer than one fourth are in stage IV.<sup>81,86,112,159</sup> Patients with lymphocyte predominance or nodular sclerosis are more likely to have stage I or II disease than are patients with mixed cellularity or lymphocyte depletion (Table 50-8). Using the Rye classification and following extensive staging procedures, among 340 consecutive patients Kaplan found 26% to be in stage I, 35% in II, 33% in III, and 6% in IV.<sup>85</sup> The frequency of stage B type of symptoms increases steadily as more extensive disease is found (Table 50-8).

## Diagnosis

Diagnosis of HD requires demonstration of the characteristic histologic picture (page

**Table 50-8. Interrelation of Histologic Classification and Clinical Staging at the Time of Diagnosis in 252 Patients with Hodgkin's disease.\* (Rye Classification)**

Histologic Type	Number of Patients	% of Patients with Clinical Stage				
		I	II	III	Local Extension from Node† IV	Diffuse IV
Lymphocyte predominance	35	14	49	32	3	3
Nodular sclerosis	114	5	50	21	12	12
Mixed cellularity	54	8	37	35	7	13
Lymphocyte depletion	49	6	43	20	6	25
Total	252	7	46	26	9	13
Percentage of patients with stage B symptoms		24	39	58	100	100

\* Adapted from Musshoff<sup>112</sup>

† Extension from a node, not stage IV in newest classification (Table 50-2)

1535). While there are certain clinical and laboratory features that allow one to suspect that a lymphoma rather than other causes of lymph node enlargement is present (Chapter 40), and features such as pruritus, alcohol-induced pain, lymphopenia, and neutrophilia suggest HD rather than another form of lymphoma (Chapter 51), histologic proof is necessary. Indications for lymph node biopsy and preferential sites for biopsy were discussed in Chapter 40. Two types of diagnostic problems are encountered with some frequency; namely, patients in whom the findings in the initial lymph node biopsy are not compatible with HD but who eventually develop the disease and patients who have symptoms compatible with HD, usually fever, but in whom no palpably enlarged lymph nodes are present and the chest film shows no abnormality.

**NONDIAGNOSTIC LYMPH NODE BIOPSIES.** Although the "typical" histologic characteristics of lymph nodes in the various forms of HD (page 1535) and non-Hodgkin's lymphoma (NHL) (Chapter 51) are recognized readily, there is no single absolute histologic criterion for the recognition of malignant lymphoma.<sup>37</sup> Initial lymph node biopsies from patients who eventually develop HD or NHL may reveal "reactive hyperplasia" and, even on retrospective review, no evidence clearly indicating malignant lymphoma may be found.<sup>37</sup> Failure to make the diagnosis may be due to the fact that one is dealing with the early stage of HD or with nodes adjacent to tumor that are reacting to the tumor. Sampling errors also are possible. Normal or enlarged but nondiagnostic nodes may be interspersed with nodes containing HD.<sup>145</sup> Multiple sections from a node must be examined before the node is considered to be nondiagnostic, since minute foci of HD may be present.<sup>148</sup> When a biopsy fails to reveal the typical histologic picture, the decision to remove another node or to observe the patient at frequent intervals must depend upon the clinical state of the patient and the degree of clinical suspicion of lymphoma (Chapter 40).

*Nonspecific granulomas* may be observed in lymph nodes, liver, spleen, and marrow of patients known to have HD.<sup>19,80,103</sup> In some patients, coexistent sarcoidosis has been considered<sup>35</sup> and, in fact, an increased frequency of sarcoidosis in association with HD has been suggested,<sup>19</sup> but, in other cases, no ancillary evidence to suggest sarcoidosis was found.<sup>19,80,103</sup> Usually it is probably best to assume that such granulomas are reactive rather than representing an early stage of HD invasion, but long-term follow-up will be required to determine if this assumption is correct.

*Reed-Sternberg cells*, while necessary to make a firm diagnosis of HD<sup>82</sup> (page 1535), are not specific for this condition. Cells resembling Reed-Sternberg cells have been observed in lymph node biopsy specimens from patients with infectious mononucleosis,<sup>106,108,150</sup> patients thought to have Burkitt's lymphoma,<sup>166</sup> following treatment of patients thought to have various types of NHL,<sup>149,150</sup> and from patients with carcinoma of the breast or lung, malignant melanoma, malignant fibroxanthoma, benign thymoma, multiple myeloma, or myositis.<sup>150</sup>

#### Patients without Palpable Disease

In some patients with HD, a mediastinal or hilar mass may be noted on routine x-ray examination of the chest or x-ray examination may be undertaken because of symptoms from such a mass. Other patients may consult the physician because of fever, fatigue, weight loss, pruritus, or other constitutional symptoms. In a minority of both types of patients no palpably enlarged nodes are available for biopsy. When there is mediastinal disease, obtaining a biopsy specimen by use of a mediastinoscope<sup>132</sup> is a much less traumatic experience for the patient than is thoracotomy. In patients who have symptoms suggestive of HD but no palpable nodes and whose chest x ray shows no abnormality, bipedal lymphangiography should be done. If this gives positive findings and/or if the spleen is palpable, diagnostic exploratory

laparotomy is advisable unless a closed needle biopsy of the liver establishes the diagnosis. If the liver biopsy and the lymphangiogram reveal no abnormality and if the spleen is not enlarged, HD is unlikely as a cause of the patient's symptoms (Chapter 40).

*Hydantoin derivatives* used as anticonvulsants may produce lymphadenopathy that cannot be distinguished histologically from HD or other forms of lymphoma.<sup>86</sup> In most circumstances, lymphadenopathy regressed when use of the drug was stopped, but in other patients the presence of true HD or NHL was established. Whether HD or NHL occurs with greater frequency in patients receiving anticonvulsants than in those not receiving such drugs is not entirely clear. If a biopsy has been compatible with a diagnosis of lymphoma in a patient in whom such medications have been employed, therapy for lymphoma should be delayed until it can be ascertained if discontinuing the use of the anticonvulsant leads to regression.

Other benign conditions that may lead to enlarged nodes with histologic changes similar to those of HD include lymphadenitis following smallpox vaccination,<sup>62</sup> herpes zoster, and toxoplasmosis.<sup>86</sup>

Hodgkin's disease beginning in the thymus is easily distinguished from primary *thymic tumors* such as the protoplasmic, small-cell, or spindle-cell type,<sup>84</sup> but there is some controversy whether *granulomatous thymoma* is or is not the same disease as nodular sclerosing HD of the thymus.<sup>50,87</sup> In some patients, later spread of disease histologically indistinguishable from HD to other parts of the body constitutes the best evidence that the disease is primary nodular sclerosing HD of the thymus.<sup>50,87,114a</sup>

## Prognostic Variables

The following factors have been well documented as having prognostic significance at the time of diagnosis: (1) clinical extent of disease—the less extensive the disease, the better the prognosis; (2) histologic type—lymphocyte predominance and nodular sclerosis imply a better prognosis than does

mixed cellularity which, in turn, is a more favorable histologic pattern than lymphocyte depletion; (3) sex—females live longer with HD than do males; (4) age—patients over 30 have a poorer prognosis than younger ones; and (5) systemic symptoms—patients with symptoms such as fever, night sweats, and weight loss have a poorer prognosis than those without these symptoms. All of these factors appear to be interrelated in part, but some are clearly independent prognostic variables.<sup>17,23,35,92,100,129,156</sup>

Lymphocyte predominance and nodular sclerosis primarily are associated with localized (stages I and II) disease, while lymphocyte depletion is associated with more widespread disease (Table 50-9). Thus, as anticipated from this association, prognosis for survival is best in patients with lymphocyte predominance and nodular sclerosis and worst in those with lymphocyte depletion (Table 50-9). However, there is some evidence that, even within clinical stages, histologic sub-type may have importance. For example, analysis of 377 patients showed that within stages, I, II, and III, patients with lymphocyte depletion tended to survive for a shorter time, whereas, within stage III<sup>105</sup> or IV,<sup>102</sup> patients with lymphocyte predomi-

**Table 50-9. Relationship of Histologic Classification to Clinical Classification and Survival\***

Histologic Type (1220 Patients)	Percent of Each Type† with Clinical Stage I or II	Percent of Each Type Surviving Five Years
Lymphocyte predominance, 13%	89%	66%
Nodular sclerosis, 40%	71%	56%
Mixed cellularity, 32%	48%	30%
Lymphocyte depleted, 16%	33%	16%

\*Based on seven series of patients reviewed by Butler.<sup>23</sup>

†Based on three of the seven series totaling 439 patients.

nance tended to survive longer than did those with other histologic types.<sup>102</sup> Mixed cellularity as well as lymphocyte depletion imply a poorer prognosis even if there is only stage I or stage II disease than does lymphocyte predominance or nodular sclerosis.<sup>88,105</sup>

Age and sex influences may not be independent variables.<sup>156</sup> The incidence of nodular sclerosing HD is higher in young than in old persons and in women than in men.<sup>113</sup> Whether the excess of nodular sclerosis fully explains the prognostic advantage of the young female requires further study.<sup>129</sup>

A "B" classification implies a poorer prognosis than "A" in any of the four numerical clinical stages. Weight loss and fever are the symptoms with the gravest prognostic significance; night sweats have some importance, but pruritus has no apparent prognostic implication.<sup>156</sup> The importance of symptoms is such that in stage III the decision to attempt curative radiotherapy is often based on the absence of symptoms. Why HD produces fever, weight loss, or pruritus is unknown.

Anemia, neutrophilia, and lymphopenia are correlated to some degree with stage of disease (Table 50-6); they may have no independent prognostic implications. The erythrocyte sedimentation rate (ESR) is not well correlated with extent of disease, but in patients without anemia or systemic symptoms an elevated ESR may indicate a relatively poor prognosis.<sup>156,162</sup>

The possible prognostic implications of such factors as anergy are still under study. Anergy usually is present in patients with advanced disease, but to date there is no evidence that it has any predictive value per se.<sup>34,170</sup> The anatomic site of lymph node disease may prove to be important (page 1541).

## Course of Disease

The course of HD depends upon the quality of response to therapy and the apparent degree of "malignancy" of the tumor, interrelated factors which can be predicted in part by the criteria just discussed.

## Duration of Survival

Median duration of survival, a figure often used in discussing the leukemias (Chapters 47 to 49), has little meaning in a disease such as HD in which a significant proportion of patients may be cured. However, in a series of 137 patients studied by us<sup>100</sup> before curative therapy was considered possible, median survival was more than 10 years in stage I, seven years in II-A, three years in II-B, and two years in stages III and IV (staging limited to physical examination and chest x ray in most cases). Survival probably is prolonged by "non-curative" radiotherapy and chemotherapy,<sup>3,129</sup> although this is somewhat difficult to prove<sup>118</sup> since there are no reports of survival in large series of untreated patients.<sup>100</sup> More than half of all patients with stage I or II disease should survive for more than five years if histologic study shows lymphocyte predominance or nodular sclerosis, but survival may be shorter if mixed cellularity or lymphocyte-depleted disease is present (Table 50-9).

## Definition of Cure<sup>33</sup>

Easson and Russell<sup>43</sup> found that, in patients who were treated by irradiation and who remained free of recurrence for a period of approximately 10 years after the diagnosis has been made, the death rate from all causes could not be distinguished from the death rate of the normal population. Thus, they suggested that cure could be defined in terms of disease-free interval following initial therapy. Further analysis seems to support the general validity of this as a general concept.<sup>33</sup> However, there is no known duration of relapse-free interval that affords a given patient a complete guarantee that disease will never recur.<sup>29,84</sup> Instead, as each relapse-free year accrues the probability of relapse in the coming year diminishes (Fig. 50-10). After five years of disease-free interval there is approximately a 1%/year possibility of relapse. These data were compiled from various institutions<sup>33</sup> on the basis of patients considered to have stage I or II disease who had



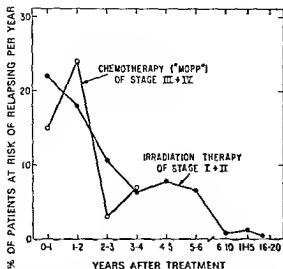


Fig 50-10. Probability of relapse in successive years following therapy with irradiation or four-drug combination chemotherapy (From data of combined irradiation series<sup>53</sup> and DeVita and Carbone's original series of patients treated with "MOPP"<sup>59</sup>)

been treated by radiotherapy. Admittedly, accuracy of staging may be questioned, particularly for the group with the longest follow-up. In certain small series of carefully staged and vigorously treated patients, such as those of Kaplan<sup>54</sup> and Musshoff,<sup>112</sup> relapse has not been observed in those at risk during the fifth through eighth disease-free years. A relapse rate curve similar to that following radiotherapy was noted in a small group of subjects treated with combination chemotherapy (Fig. 50-10). Although for the present, an exact definition of cure cannot be given, a disease-free interval of four to five years following therapy provides a reasonable statistical probability that a cure has been achieved.<sup>53,84</sup> By this definition, cure is anticipated in a significant percentage of patients treated by radical radiotherapy (page 1556) and it is possible, although not yet proven, that a number of patients with advanced disease may be cured by four-drug combination chemotherapy (page 1559).

#### Course in Patients Who Are Not Cured

Even when disease cannot be completely eradicated, repeated complete or partial remissions can be induced, even though even-

tually the disease cannot be controlled and death occurs. Pre-terminal, uncontrolled disease may not be characterized by formation of large, bulky tumors; often, in fact, it is characterized by minimal palpable lesions and yet there is considerable fever, weight loss, and consequent cachexia. Even at autopsy there may be no evident disease in nodes.<sup>69,91</sup> Formation of troublesome pleural or peritoneal serous effusions is not uncommon.<sup>161</sup> Jaundice is observed at some time during the course of disease in approximately 20% of patients and while it is most commonly accounted for by HD invasion of the liver, a diverse number of causes is possible which are difficult to distinguish from one another unless a biopsy specimen of the liver is obtained<sup>98</sup> (Chapter 54). A variety of peculiar and poorly understood complications such as *nephrotic syndrome*<sup>126</sup> may develop during the course of the disease (Chapter 54). Even with aggressive therapy, complete control of the disease is not achieved, even for a short time, in a few unfortunate patients; in many of these the histologic picture is that of lymphocyte depletion.<sup>112a</sup> Other patients appear to have an innately less aggressive disease. Patients have been described who lived for 26 and even 33 years but were never clearly in complete remission throughout the entire course of the disease.<sup>27</sup> As noted by the occasional relapses after many years free of disease following therapy (page 1554), HD may remain dormant for protracted periods; alternatively, perhaps such cases represent re-induction of disease rather than re-growth of the original tumor.

The cause of death is quite variable. Many patients die with increasing fever and cachexia in the absence of a single identifiable cause of death. Hepatic or pulmonary failure secondary to invasion by HD may lead to death (Chapter 54). Tuberculosis, toxoplasmosis, or severe systemic herpes virus infection<sup>25</sup> may develop as a result of anergy, or terminal pyogenic bacterial infection may occur in association with tumor invading the lung or following neutropenia and immune depression resulting from therapy (Chapter 54). Some degree of bone marrow failure,

reflected in pancytopenia or in the prompt appearance of pancytopenia following small amounts of therapy is fairly common late in the disease. This may be the result of HD and fibrosis in the marrow, unrepaired damage from irradiation and chemotherapy, or combinations of these events. A wide variety of complications such as multifocal leukoencephalopathy have been the cause of death (Chapter 54)

## Therapy

Cure of disease constitutes the proper goal of therapy in *all* previously untreated patients with HD and even in those who have relapsed following curative attempts with radiotherapy.

*Palliation* is presently the only practical goal in patients in whom relapse occurs after an attempt to cure the disease with radiotherapy followed by chemotherapy, or in those in whom chemotherapy was employed as the initial curative measure. Defining the goal as cure or palliation is a decision which must be made because attempts to cure with radical irradiation therapy or with four-drug combination chemotherapy are associated with sig-

nificant morbidity and even occasional mortality. Such morbidity induced by therapy must be kept to a minimum if only the goals of palliative therapy (Chapter 55) are to be attained.

Although on rare occasions cures have followed simple surgical excision, attempts to cure by radical node dissection have been unsuccessful<sup>1</sup> on the whole. Spontaneous remissions, however, have been reported.<sup>116</sup>

## Radical (Curative) Radiotherapy

Although it had long been known that a few patients were apparently cured following radiotherapy<sup>169</sup> or even chemotherapy<sup>100</sup> and some radiotherapists had advocated "sterilization" of lesions,<sup>54</sup> it was the studies of Peters<sup>123</sup> and Easson and Russell<sup>43</sup> that provided the first evidence that cure is a reasonable goal of radiotherapy. Kaplan<sup>92,93</sup> developed scientific guidelines for achieving this goal by studies in which it was shown that the likelihood of recurrence of disease at an irradiated site was less than 5% if 3500 rads or more were delivered to that site within a period of approximately four weeks (Fig. 50-11); recurrence was only about 2% if 4000 rads were given.<sup>96</sup> Delivery of this

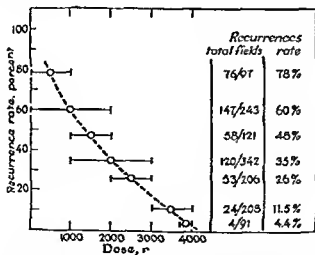


Fig 50-11 Rate of recurrence of Hodgkin's disease in a given treatment field as a function of dose of irradiation delivered to that field (From Kaplan,<sup>92</sup> courtesy of the author and Cancer Research)

amount of irradiation in that short a time to a tumor requires the use of the therapy machines developed during the last two decades (see Chapter 55). "Conventional" 250-kilovoltage x-ray treatment machines produce intolerable skin damage that is largely avoided by the use of high-energy rays generated by megavoltage linear accelerator, telecobalt, or betatron apparatus.<sup>86</sup> However, kilovoltage therapy is still utilized and advocated by some.<sup>68</sup>

Thus, cure can be anticipated if 3500 to 4000 rads are delivered to *all* existing tumors within four weeks. The total dose may be more important than the delivery interval since a high cure rate has been reported if 4000 rads was delivered in six rather than three to four weeks.<sup>77</sup> The probability of cure in each treatment field appears to be an independent variable.<sup>86</sup> Thus, if the probability of cure of a single area is 98% with 4000 rads, the probability will decrease to 92% (.98<sup>3</sup>) if three areas are involved. Similarly the probability of cure of a single area with 3500 rads will decrease from 95% to 86% if three areas are involved. For this reason the total dose given to involved areas may be more critical when multiple sites are involved than when a single site is affected.

Irradiation of areas adjacent to and in the probable natural line of spread of disease from areas of known involvement has been advocated (*extended field irradiation*). It seems reasonable to suspect that inapparent spread has occurred to adjacent areas in some patients. Thus, most would agree that "mantle" therapy is indicated for stage I or II disease above the diaphragm and the "inverted Y" for stage I or II disease below the diaphragm (Fig. 50-12A and B). If the spleen has not been removed it should be irradiated in addition to the inverted Y. All areas are given 3500 to 4000 rads within four weeks, if possible. Following the same reasoning it has been argued that the para-aortic area (Fig. 50-12C), or even the entire lymph node chain (Fig. 50-12D), should be irradiated even in patients with stage I supradiaphragmatic disease.<sup>85</sup> However, it is generally agreed that, if stage III disease is present, "total nodal" irradiation (Fig. 50-12D) should be given. This term is a misnomer, however, in the sense that many peripherally located lymph nodes are not treated.

Although the reasoning behind extended field irradiation appears sound and reported results appear quite good in some instances, it must be emphasized that the case for its

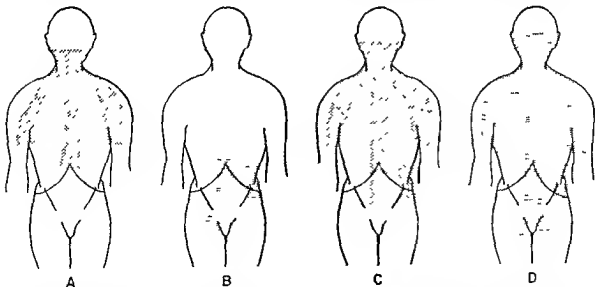


Fig. 50-12. Treatment fields employed with extended field irradiation therapy, the mantle (A), the inverted Y (B), mantle and para-aortic (C), and "total nodal" (D). The spleen is irradiated in conjunction with the inverted Y unless it has been removed.

use has not been proved.<sup>17,92</sup> A randomized comparison of limited versus extended field therapy was stopped when it appeared that the additional irradiation resulted in a significantly longer disease-free interval. However, further analysis disclosed the fact that the two treatment groups had not been really comparable.<sup>86</sup> Nevertheless, a preliminary report of a randomized study of the mantle versus total nodal therapy in stages I and II disease suggests that total nodal therapy may be superior<sup>77</sup> (Fig. 50-13). Randomized study of the possible value of prophylactic irradiation is needed.<sup>115</sup> There is need also for continued randomized comparative studies based on increasing knowledge of the pattern of disease spread. For instance, were the case for total nodal irradiation for stages I and II disease proved desirable, is mediastinal irradiation

justified in a patient with high cervical stage I disease in view of the rarity of mediastinal disease in that circumstance?<sup>154</sup>

**Toxic effects** of irradiation with 3500 to 4000 rads are considerable. Organs such as the bone marrow, lung, heart, kidney, liver, and spinal cord may be damaged irreparably by this dose<sup>76</sup> (Chapter 55). Acute myeloblastic leukemia and CML probably have been induced in treated patients<sup>152</sup> (Chapter 46). Thus, continued comparison of series of patients treated by different regimens of radical irradiation must also include long-term comparisons of the causes and severity of morbidity and of mortality<sup>66</sup> as well as the frequency of relapse from HD. Death from irradiation-induced pulmonary fibrosis cannot be considered preferable to death from HD, and other complications (Chapter 55) such as radiation-induced pericarditis with subsequent constrictive fibropericardium<sup>112</sup> cannot be dismissed lightly.

**Shielding** of normal tissue is thus as important in attempting curative radiotherapy as is irradiation of the tumor. Insofar as is possible, all normal tissue, particularly lung, heart, bone marrow, liver, and kidneys, must be shielded from treatment fields. If an exploratory laparotomy is performed, the spleen should be removed, not only to examine it for HD, but to exclude the necessity for splenic irradiation thus sparing the left kidney and left lower lobe of the lung. Likewise, if the abdomen is explored in female patients, both ovaries should be moved laterally to remove them from the possible treatment field. Individualized lead shields must be constructed for each patient and changes in shielding may be necessary during therapy. For example, large mediastinal masses may require inclusion of much of the lungs in the original treatment field; as the tumor shrinks the treatment field should be reduced by increasing the area of shielded lung. With modern megavoltage machines and with the increasing number of hospitals in the United States that have such machines, delivering a tumoricidal irradiation dose to a patient with HD is rarely a problem. Hopefully, knowledge and technique concerning shielding will grow commensurate with proliferation of irradiation equipment.

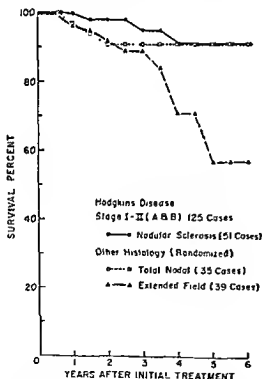


Fig 50-13 Comparison of results of treatment of patients with stages I and II Hodgkin's disease with extended field or "total nodal" irradiation. Patients were randomly assigned to treatment schedules; the extended field usually consisted of mantle irradiation. Actual survival curves are shown. The effects of the two types of treatment differed significantly except in patients with nodular sclerosis. (From Johnson et al,<sup>77</sup> courtesy of the authors and Cancer Research.)

**RESULTS OF RADICAL RADIOTHERAPY.** As many as 90% of patients with stages I and II-A HD may be cured by radical radiotherapy (Figs. 50-13 and 50-14), although some reports are not quite so promising.<sup>128,158</sup> With stages I-B and II-B the prognosis is not quite as bright, but more than half of the patients in these stages appear to be cured (Figs. 50-13 and 50-14). In patients with stage III-A, cure is achieved in somewhat less than 50% and most of those with III-B have experienced relapse within three to four years.<sup>75</sup> For this reason we and many others recommend radiation as initial therapy for patients having stages I to III-A and chemotherapy initially for those having stages III-B and IV. Alternatively, for those advanced stages, combination chemotherapy-radiotherapy can be employed (page 1561). As previously discussed (page 1553), the frequency of relapse in part depends upon pathologic classification (Table 50-10).

### Palliative Radiotherapy

In the patient in whom cure no longer seems possible, radiotherapy is still of benefit although chemotherapy is preferable for most of this group. In most patients, delivery of 1000 to 2000 rads will induce significant tumor regression and sometimes tumor disappearance. Radiotherapy may be used to rapidly reduce masses obstructing vital organs, eg, spinal cord compression, bronchial constriction, vena cava obstruction or obstruction of the common bile duct (Chapter 54). When most symptoms are thought to be due to tumor in a single site in patients still pancytopenic following chemotherapy, a modest local dose of irradiation may prove helpful. The practice of "chasing" the tumor with palliative doses of radiotherapy has been largely supplanted by chemotherapy.

### "Curative" Chemotherapy

The results of the original trial of the four-drug regimen known as "MOPP" (Table 50-11) suggest that some of the patients may have been cured.<sup>39,40</sup> Of 43 patients entered in the study by July 1967, there

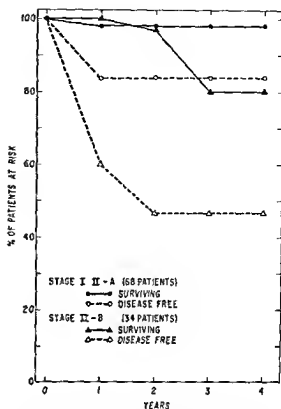


Fig 50-14. Survival and disease-free interval following radical radiation therapy (Adapted from data from Johnson<sup>75</sup>)

Table 50-10. Extranodal Relapse after "Total Nodal" Irradiation as It Relates to Pathologic Classification and the Presence or Absence of Symptoms\*

Pathologic Classification	Number of Patients with Extranodal Relapse	
	Stage I-III A	Stage I-III B
Lymphocyte predominance	0/26	0/5
Nodular sclerosis	2/40	2/13
Mixed cellularity	4/25	6/13
Lymphocyte depletion	1/6	5/5

\*Adapted from Johnson et al.<sup>77</sup> Since the duration of follow-up is quite variable, these figures cannot be equated with "cure."

**Table 50-11. A Combination Chemotherapy Regimen for Patients with Hodgkin's Disease**

Total of six courses each course beginning every 28 days\*

Each course

- M—Nitrogen mustard 6 mg/m<sup>2</sup>† on day 1 and day 7  
 Q—Vincristine (Oncovin) 1.4 mg/m<sup>2</sup> on day 1 and day 7 (should not exceed 2 mg/dose)  
 P—Procarbazine 100 mg/m<sup>2</sup> on day 1 through day 10  
 P—Prednisone 40 mg/m<sup>2</sup> on day 1 through 14, in 1st and 4th courses only

\*Frequency and intensity of each course is modified if toxicity develops

†Square meter of body surface area

were three with stage III-A disease, five with III-B, four with IV-A, and 31 with IV-B; thus extensive disease was present in all. There were two drug-related deaths. The remaining patients improved, 81% achieving complete remission (no evidence of residual disease) and, four or more years after stopping therapy, 43% of those who had achieved complete remission were still free of evident disease.<sup>24</sup> The pattern of relapse approximates that seen after irradiation therapy (Fig. 50-10). Although caution must be exercised in considering these patients as having been cured there is reason to think that this is a possibility. Results of comparable studies indicate a similar complete remission rate, but follow-up periods to date have been too short to allow assessment for the possibility of cure. Preliminary reports of trials of other drug combinations indicate that the incidence of complete remissions is no higher than with "MOPP" and relapse occurred after a short interval in a larger number of patients.<sup>159</sup> Substitution of vinblastine for vincristine in the regimen led to an apparent increase in degree of leukopenia without an appreciable increase in rate of remission.<sup>114</sup> Periodic re-induction doses of "MOPP" may delay relapse,<sup>51</sup> but whether such therapy is preferable to withholding such "maintenance" therapy and then attempting re-induction of

remission after relapse has developed is not yet known. "MOPP" therapy in patients relapsing after radiotherapy has resulted in a percentage of remissions approximating that obtained in untreated patients.<sup>21,51,101,111</sup> However, when an extensive history of chemotherapy preceded MOPP, the rate of remission was quite low, particularly when resistance to drugs in the regimen had been noted before their use in the regimen.<sup>51,101,114</sup> Radiotherapy did not appear to predispose to a significant increase in bone marrow toxicity when "MOPP" was given subsequently.<sup>24,151</sup> However, it must be kept in mind that permanent fibrosis of bone marrow has followed 3500 rads<sup>153</sup> and three to six months have generally been required for recovery of normal marrow function following irradiation.<sup>56</sup>

Thus, for the present, "MOPP" therapy appears advisable for all patients in stage IV when first treated and perhaps in III-B as well as for patients relapsing after attempted curative radiotherapy. There is no contraindication to MOPP therapy. However, contrary to the recommendation of DeVita and Carbone,<sup>39</sup> it seems reasonable to recommend reducing the dose of nitrogen mustard (HN2) and procarbazine in the presence of neutropenia or thrombocytopenia, as shown in Figure 50-15. The patient described there illustrates the dramatic results that can be obtained with combination chemotherapy. Beginning with small doses of procarbazine and HN2 and full doses of vincristine and prednisone this patient improved with each course and remained in complete remission for two years.

Not all patients are responsive to combination chemotherapy or to irradiation and complete remission is never achieved in a few.

If it should prove that a significant number of patients with stages III-B and IV are cured by combination chemotherapy, then its possible use as a substitute for radiotherapy in more localized disease will need to be considered. Consequently, the results of its use for all stages of HD where radiotherapy is not available are of special interest.<sup>117</sup> In a preliminary report of a small series of pa-



proportion of the lung must be irradiated also. One or two courses of MOPP therapy given before irradiation may reduce the mass to the point at which much of the lung may be shielded during subsequent radiation therapy. Preliminary reports of combined radiotherapy-chemotherapy suggest that "curative" radiotherapy can be followed by a full course of MOPP without intolerable toxicity occurring.<sup>109,136a</sup> On the other hand, severe hematopoietic depression was reported in one study in which intensive chemotherapy was followed by radiotherapy.<sup>65a</sup>

### Palliative Chemotherapy

Since the introduction of *nitrogen mustard* (HN2)<sup>164</sup> a number of chemotherapeutic agents that are useful in the treatment of HD have been developed.<sup>12,158</sup> (Chapter 55). These can be classified as (1) alkylating agents such as HN2,<sup>19,71</sup> chlorambucil,<sup>48</sup> and cyclophosphamide<sup>71</sup>; (2) the vinca alkaloids, vinblastine (VLB)<sup>6</sup> and vincristine (VCR)<sup>25</sup>; (3) the methylhydrazine derivative procarbazine<sup>18</sup>; (4) adrenocorticosteroids<sup>59</sup>, and (5) agents still considered experimental such as bleomycin, BCNU,<sup>96</sup> and streptozincin.<sup>57</sup>

The frequency of improvement with most of these agents when used singly is quite similar (Table 50-12). Complete remission is much less common than following combination chemotherapy, although apparent "cures" have been reported.<sup>90</sup> The quality of response depends, at least in part, on the dosage employed. Maximally tolerated doses of drugs used as single agents produce a higher frequency of response than do smaller doses, but at the expense of increased morbidity due to drug toxicity.<sup>52</sup> In general, response to a new agent in patients who have received extensive prior chemotherapy is less good than occurs when that drug is used early in the course.<sup>52</sup> Whether this is due to lower drug tolerance or to intrinsically more resistant disease is not entirely clear. However, in a controlled crossover type of study, response to VLB and HN2 was the same regardless of the order in which they were used.<sup>6</sup> Remissions following the use of single agents usually are short without additional

**Table 50-12. Remission Rate Following Combination or Single Agent Chemotherapy\***

Therapy	Number of Patients Evaluated	Improved (%)	Complete Remission† (%)
Combined			
HN2			
Procarbazine			
Prednisone			
Vincristine	124	—	69
("MOPP")			
HN2	760	63	13
Cyclophosphamide	469	54	12
Chlorambucil	305	60	16
Vinblastine	705	68	30
Vincristine	105	64	36
Procarbazine	366	69	38
Prednisone	105	61	0
BCNU	149	50	5

\*From data reviewed by Carter and Livingston.<sup>24a</sup>

†Complete remission was defined by stricter criteria in the group receiving combination chemotherapy than in most groups receiving single-agent therapy. Thus, the difference between percentage in complete remission following combined versus single agent therapy is probably underestimated.

therapy, but they can be maintained for some time by continuing the use of the drug (Table 50-13). Selected dosage schedules of various drugs used as single agents are presented in Table 50-14.

*Drug resistance* is judged to be present when less than 50% tumor regression occurs with standard dosage or when the tumor begins to grow while the patient is on full-dose maintenance therapy. Larger doses may still result in reduction of tumor, but bone marrow toxicity increases commensurate with the increased dose and may become prohibitive. Resistance to one alkylating agent usually signifies resistance to all alkylating agents, but whether there is cross resistance between VCR and VLB is less certain. Resistance to alkylating agents and VLB does not imply resistance to procarbazine.<sup>38</sup>

It is now a common practice to initiate chemotherapy with "MOPP" and to use this until the patient proves resistant. Consequently, the drugs available to the "MOPP"-resistant patient are limited to VLB or experimental agents such as bleomy-



**Table 50-13. Other Results of Therapy with Drugs Used Singly\* and Duration of Remissions in HD**

Drug	Number of Patients	Percent Improved†	Duration of Response (Mean)	
			Therapy Unmaintained (Months)	Therapy Maintained (Months)
Nitrogen mustard <sup>39</sup>	215	61	2.5	
Supplemented by chlorambucil maintenance <sup>71</sup>	19	58		10.0
Cyclophosphamide <sup>19,71</sup>	441	59	3.0	8.0
Vinblastine <sup>39</sup>	551	64	2.0	6.5
Vincristine <sup>25</sup>	10	80		4.0
Procarbazine <sup>38</sup>	573	75		4.0
BCNU <sup>39</sup>	31	55		> 4.0
Prednisone <sup>39</sup>	24	75		2.0

\*From collected series<sup>39</sup> or from references as shown for each drug

†Measurable tumor regressed at least 50%

cin, BCNU, and streptomyacin (Chapter 55). Prednisone still may be useful in the "MOPP" resistant patient since it is given for a short time only in the "MOPP" regimen (Table 50-11).

During symptomatic therapy it is important to keep in mind the general principles of such therapy. These emphasize the objectives of keeping the patient as comfortable as possible (Chapter 55). Thus, if the patient

**Table 50-14. Suggested Dosage Schedule for Single-Agent Therapy in Hodgkin's Disease**

Agent	Daily Dose	Frequency of Dose	Duration
Nitrogen mustard	0.3–0.4 mg/kg iv or 0.1 mg/kg/day for 4 days <sup>117</sup>	Two consecutive doses every 4–6 weeks	Until all evidence of disease disappears or drug resistance supervenes
Chlorambucil	0.1 mg/kg, orally	Daily	Until all evidence of disease disappears or drug resistance supervenes
Cyclophosphamide	2 mg/kg, orally or 40 mg/kg as single iv dose	Daily	May be continued at reduced dose as maintenance therapy
Vinblastine	0.15 mg/kg iv (increase until leukopenia occurs)	Weekly	Relapse occurs quickly if therapy is stopped; interval between doses can be lengthened
Vincristine	1.4 mg/m <sup>2</sup> (not to exceed 2 mg total)	Once a week	Repeat dosage until resistance appears or toxicity develops
BCNU	100 mg/m <sup>2</sup> daily X 2, iv	Every 4–6 weeks	Repeat dosage until resistance appears or toxicity develops
Prednisone	60 mg/day	Daily	Stop in 4–6 weeks

is asymptomatic or relatively so, no therapy may be advisable. In this case the presence of palpable HD does not in and of itself demand therapy since eradication of the disease is no longer considered possible. If drugs are considered for use in doses calculated to induce toxicity, this must be done with the anticipation that the symptomatic benefit to be achieved will more than counterbalance the period of increased discomfort induced by therapy. Adjunctive therapy (Chapter 54) is quite important in palliation; eg, control of fever by indomethacin<sup>143</sup> may improve the patient's well-being significantly.

**INADEQUATE INITIAL THERAPY.** Unfortunately many patients with HD are not being staged carefully and still receive palliative radiotherapy or chemotherapy at the time of diagnosis. Still others with relatively slowly progressive disease were treated in this fashion some years ago and are only now showing signs of relapse. Deciding how to manage such patients constitutes a difficult problem. There are no data to suggest that curative radiotherapy can be given safely and successfully to patients relapsing from an initial course of irradiation. It seems reasonable, however, to treat such patients with "MOPP."

*Patients in whom no modality of therapy proves to be of much benefit remind us that while the outlook for the patient with HD has improved, the problem is by no means solved.*

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## Lymphomas Other than Hodgkin's Disease

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- Histologic Patterns
- Mode of Presentation
- Physical Findings
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- Diagnosis and Differential Diagnosis
- Course and Survival
- Causes of Death
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- Less Common Lymphomas
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- Histiocytic Medullary Reticulosis

THE non-Hodgkin's lymphomas (NHL) represent the malignant tumors of lymphoid tissue, excluding Hodgkin's disease (Chapter 50) and the lymphoid leukemias (Chapters 47 and 49). They can occur in individuals of any age although they are exceedingly rare in children younger than two years<sup>109,113</sup>, all types become increasingly frequent as age advances (Chapter 46). These lymphomas are more common in males than in females (Chapter 46).

### Classification

Since the non-Hodgkin's lymphomas were first recognized as a distinct group (Chapter 46)<sup>46,59,103,109</sup> a variety of classifications have been proposed but none is entirely satisfactory. The cells that infiltrate the nodes and other tissues in NHL are forms of lymphocytes and histiocytes ("reticulum" cells), but the degree of differentiation of these cells as judged by histologic criteria differs from patient to patient; the cell type may be relatively uniform or a mixed population may be present and the cells may be arranged in a diffuse or in a nodular (follicular) pattern. The ideal classification of the NHL would be reproducible in the judgment of different observers and each subclass would be expected to differ from the others with respect to clinical manifestations, response to therapy, and prognosis. Three schemes of classification in particular have enjoyed some popularity—those of Craver,<sup>109</sup> Gall and Mallory,<sup>59</sup> and Rappaport<sup>103</sup> (Table 51-1). That of Rappaport is the most recent and is now in vogue, but earlier classifications must be understood in order to appreciate much of the earlier literature.

The classification popularized by Craver and others<sup>109</sup> was based on a division of NHL into three broad categories—lymphocytic lymphosarcoma (LLSa), reticulum cell sarcoma (RCSa), and giant follicle lymphosarcoma (GFLSa). The advantages of this

**Table 51-1. Alternative Classifications of Non-Hodgkin's Lymphoma and the Approximate Relationships of the Various Groups**

Craver <sup>109</sup>	Gall and Mallory <sup>89</sup>	Rappaport <sup>105</sup>
Lymphocytic lymphosarcoma (LLSa)	lymphocytic lymphoma	lymphocytic well differentiated
Reticulum cell sarcoma (RCSa)	lymphoblastic lymphoma stem cell lymphoma clasmatocytic lymphoma	lymphocytic poorly differentiated undifferentiated histiocytic differentiated mixed lymphocytic histiocytic
Giant follicle lymphosarcoma (GFLSa)	follicular lymphoma	follicular (nodular) (this term is added to the primary designation of any of above types in which a follicular pattern predominates)

classification are its simplicity and the demonstrated clinical differences between the three subtypes. Its drawbacks include the fact that, in GFLSa, the type of cells making up the giant follicles are not uniform and in later stages the follicular structure may be lost. Less striking degrees of follicle formation may be present in the other categories of lymphoma and their clear separation from GFLSa may therefore be difficult. As to RCSa, cases not fitting LLSa or GFLSa often have been classed as RCSa. As discussed in Chapters 6 and 8, the term "reticulum cell" is not a very satisfactory one.

Gall and Mallory<sup>89</sup> suggested that five categories of disease could be recognized—lymphocytic, lymphoblastic, stem cell, clasmatocytic, and follicular lymphoma. They reviewed the records of 389 patients and searched for clinical differences between the different types. A number of minor differences of questionable significance were noted, but the major ones related to duration of survival, spleen size, and leukemic conversion. Follicular disease was associated with the longest survival, followed by lymphocytic and, in turn, by stem cell, clasmatocytic, and lymphoblastic lymphoma. There was little difference in survival rates among subjects having the last three pathologic types. Leukemic conversion was most common in patients with the lymphocytic variety; un-

common in those with the stem cell, clasmatocytic, or follicular types; and intermediate in incidence in those having the lymphoblastic form. Splenomegaly was encountered most frequently in patients with the lymphocytic type, and in descending frequency in those with the lymphoblastic, follicular, clasmatocytic, and stem cell types. Thus, there were clinical as well as pathologic differences in Gall and Mallory's five categories, but differentiation of the lymphoblastic-stem cell-clasmatocytic types was on less solid clinical grounds than the separation of these from GFLSa and LLSa. In another study,<sup>90</sup> undifferentiated RCSa was found to be similar to histiocytic RCSa in most respects, including prognosis, but was more likely to undergo leukemic conversion and less likely to be associated with skin involvement.

Rappaport and associates<sup>106</sup> proposed that the nodular (follicular) varieties of disease be subdivided into distinct cell types rather than considering GFLSa as an entity. The distribution of cases classified according to the Rappaport classification as compared with that of Craver is shown in Table 51-2. They pointed out that a nodular or a diffuse pattern may be seen in each of five cellular types, which they named well-differentiated lymphocytic, poorly differentiated lymphocytic, undifferentiated, histiocytic differentiated,

and mixed lymphocytic-histiocytic. A nodular pattern is most commonly observed in the poorly differentiated lymphocytic (Fig. 51-2) or mixed cell types.<sup>75</sup> In all varieties of NHL, a follicular pattern implied a better prognosis than did a diffuse one,<sup>106</sup> confirming the original observations of Brill and co-workers<sup>25</sup> and Symmers.<sup>129</sup> Survival was found to be distinctly superior in the well-differentiated lymphocytic-follicular type as compared with the other follicular varieties.<sup>106</sup> However, this has not been the experience of some other investigators.<sup>104</sup>

The Rappaport classification is being adopted by many institutions, but whether all of the categories will prove to be useful remains to be determined. In the study of clinicopathologic correlations in 223 patients summarized in Table 51-2,<sup>75</sup> differences between some, but not other, categories were apparent. Any degree of nodularity was found to confer a better prognosis than was noted with any diffuse variety (Table 51-3). Nodular forms were uncommon in children and young adults as compared to their occurrence in older patients, and they tended to be more common in males than in females. Extra-lymphatic spread, as well as systemic symp-

toms, was more common in patients with the diffuse types than in those having the nodular varieties. With regard to differences between lymphocytic, mixed, and histiocytic varieties (within either diffuse or nodular categories), patients with the lymphocytic and mixed forms survived longer than those with the histiocytic forms. Bone marrow involvement was more common in those with the lymphocytic type.

In a patient with NHL, when the pathologic picture changes with time, the change usually is toward a more undifferentiated form of disease, as from LLSa to RCSa,<sup>38</sup> or GFLSa to LLSa or RCSa.<sup>20</sup> Gall and Mallory<sup>59</sup> noted a changing pathologic picture in 23% of 84 patients studied serially, the change being always toward a more undifferentiated picture. In patients with follicular disease, approximately one half lose the follicular pattern and acquire a lymph node picture of diffuse, unstructured cellular infiltration at death.<sup>106</sup> Conversion from a pathologic picture of any form of NHL to a picture of Hodgkin's disease or vice versa is most unusual, if it ever occurs.<sup>59,103</sup>

In general, in adults, LLSa, probably is the most common disease form, being more

**Table 51-2 Relation of Craver Classification to Rappaport Classification in 223 Reclassified Cases**

Rappaport Classification	n*	Craver Classification			
		RCSa	LLSa	GFL	UC†
Nodular (total)	98	28	20	47	3
Histiocytic	15	10	1	2	2
Mixed	44	11	10	22	1
Lymphocytic poorly differentiated (LLPD)	38	7	8	23	0
Lymphocytic well differentiated (LLWD)	1		1	—	—
Diffuse (total)	125	91	33	1	0
Histiocytic	68	65	1	0	—
Mixed	15	10	5	—	—
LLPD	30	12	17	1	—
LLWD	7	0	7	0	—
Undifferentiated	7	4	3	—	—

\*Values are number of patients

†Unclassified

Modified from Jones et al<sup>75</sup>



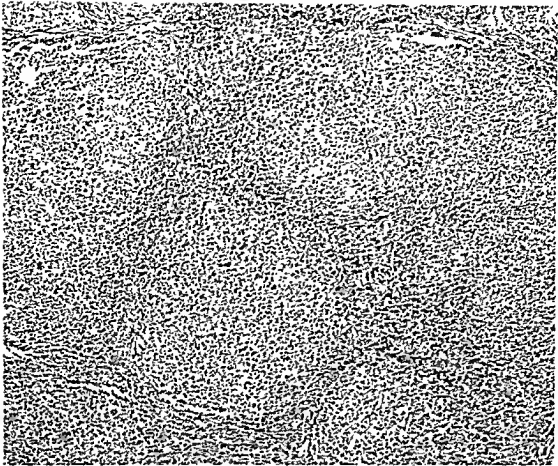


Fig 51-1. Lymphocytic lymphoma, poorly differentiated nodular type. Immature lymphocytes are arranged in nodules which expand to compress the adjacent more darkly staining nodal components. (Lymph node H & E stain 100 $\times$ ) (Courtesy of Dr R W McDwitt)

common than RCSa in some studies<sup>45,67</sup> and about equally common in others<sup>109</sup>; GFLSa is least common, making up approximately 10% of all NHL<sup>67,109</sup> or even less.<sup>45</sup> In chil-

dren, RCSa, especially the lymphoblastic variety, is more common than LLSa.<sup>7</sup> While GFLSa may be observed in children,<sup>7,20</sup> it makes up an even smaller proportion of the cases than in adults.<sup>109</sup>

Table 51-3. Relation of Histologic Pattern to Duration of Survival

Cell type	Pattern	
	Nodular (years)	Diffuse (years)
Histiocytic	3.5	1.3
Mixed	8	2
LLPD	9	2
LLWD	-	-
Undifferentiated	-	0.5

\* Long survival (> 9 years) but too few cases to calculate accurately

Based on data of Jones et al<sup>75</sup>

## Histologic Patterns of Non-Hodgkin's Lymphoma

Destruction of the normal nodal architecture, the presence of pseudo-follicles, invasion of the capsule with extension into the adjacent fat, and the presence of neoplastic cells either in the pulp, the sinuses, or within pseudo-follicles are the usual criteria for histologic diagnosis in excised lymph nodes. Portions of the node may appear normal. As noted elsewhere (Chapters 40 and 50), in

patients who eventually develop malignant lymphoma the initial biopsy specimen or specimens may be reported as representing "reactive hyperplasia,"<sup>43</sup> perhaps because the specimen is from a non-involved node.

*Well-differentiated lymphocytic lymphoma* is characterized by the presence of cells that resemble the normal small lymphocyte. The infiltration is quite uniform and there are few mitotic figures. The uniform cellular infiltration obscures nodal architecture, but the nodal capsule may be intact.

*Poorly differentiated lymphocytic lymphoma* (lymphoblastic lymphosarcoma), the most undifferentiated form of which is included under RCSa in the Craver classification, is made up of cells that do not conform entirely to the characteristics of a lymphoblast, as seen in the blood of patients with lymphoblastic leukemia (Chapter 47). The cell is 10 to 20  $\mu$ m in diameter. The nucleus is centrally placed and round or slightly indented and possesses a distinct border. It is made up of chromatin that is evenly distributed and less clumped than in a lymphocyte. Nucleoli may or may not be evident. There is a uniformly narrow basophilic rim of cytoplasm, spherical in outline or irregular. Mitotic figures may be seen.

*Undifferentiated and differentiated forms of histiocytic lymphoma* are distinguished. In the *undifferentiated or syncytial form*, the predominating cell is relatively large (diameter of 15 to 35  $\mu$ m) with varying amounts of cytoplasm that frequently is scanty and pale staining. A syncytial arrangement often prevails. The nucleus is vesicular and is about two to four times as large as that of a lymphocyte. It is round or oval in shape or slightly indented. The chromatin appears delicate; it is irregularly distributed and lacks points of condensation. There is a thin, but distinct, nuclear membrane and a single, prominent, dark-staining nucleolus. Reticulum fibers are scanty and are mostly limited to preexisting stroma. There is little evidence of histiocytic or lymphocytic differentiation. The *differentiated or histiocytic form* (Fig. 51-2) is composed predominantly of neoplastic histiocytes that possess the power of

phagocytosis, fibril formation, or both. The cells are smaller than in the undifferentiated form, but are larger than lymphocytes, being 15 to 20  $\mu$ m in diameter. The nucleus often is eccentric; it may be round but more often is oval, reniform, or horseshoe-shaped. The chromatin is fine and nucleoli are rare. The cytoplasm is abundant, generally eosinophilic, and the borders tend to be irregular in outline, suggesting ameboid propensities. Engulfed particles and sometimes whole cells may be found in the cytoplasm.

*Malignant lymphomas of the mixed cell type* are characterized by a neoplastic proliferation of histiocytes and lymphocytes without appreciable predominance of either cell type. These often exhibit a follicular pattern.<sup>108</sup>

Any of the above-mentioned cellular types of NHL may assume a follicular pattern. The diagnosis of *follicular or nodular lymphoma* rests on the presence of large pseudo-follicles rather than upon a specific cell type (Fig. 51-3); any residual normal glandular tissue is compressed by the large and often confluent follicles. Trabeculae are obscured and there is a stromal rearrangement unlike that seen in normal lymph node hyperplasia. The follicles are larger and more numerous than those usually observed in simple hyperplasia. Their close-packed arrangement, their relatively uniform size, together with their distribution throughout the node, rather than being limited to the cortex, and the condensation of the reticulum, help to distinguish them from what is seen in simple hyperplasia. In some series,<sup>73</sup> any degree of nodularity, even if it involves only part of a node, and even very subtle nodularity requiring special stains to demonstrate "nodules" delineated by compressed reticulum fibers surrounding them, have been considered sufficient to classify the lymphoma as follicular.

Differentiation of benign and lymphomatous infiltrations can be quite difficult (pages 1552 and 1585, Chapter 40). This is particularly true of well-differentiated NHL with or without a follicular pattern. First and two other pathologists reviewed material from 40 patients in whom the original diagnosis was GFLSa, but all three were in

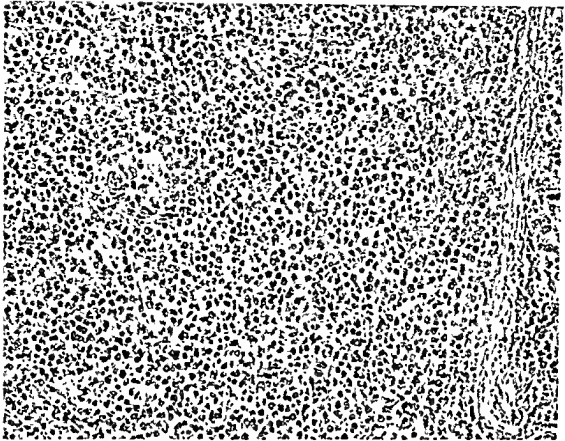


Fig. 51-2. Histiocytic lymphoma, diffuse type. Nodal architecture is obliterated by a diffuse infiltration of histiocytes (Lymph node H & E stain 160 $\times$ ) (Courtesy of Dr. R. W. McDivitt)

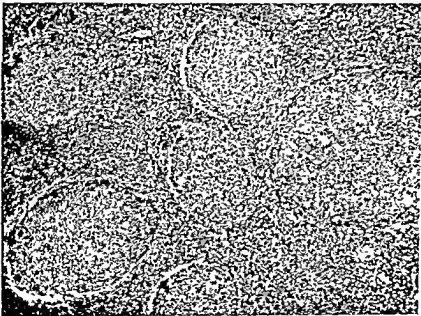


Fig. 51-3. Follicular lymphoma. Lymph node ( $\times 60$ ) (Mayer and Thomas,<sup>90a</sup> courtesy of Bulletin of Johns Hopkins Hospital)

agreement in only 22 instances.<sup>56</sup> They also noted that "considerable variation occurred in the check sheets from day to day when the same pathologists reviewed the same slides."

The relative frequency of the various histologic types of NHL is shown in Table 51-4.

## Mode of Presentation

Most patients with NHL are first seen with disease primarily in lymph nodes. The most common initial complaint is enlarged lymph nodes. Generally there are no systemic symptoms. The nodes usually, but not invariably, are painless and often have been noted in cervical or supraclavicular areas. Fatigue, malaise, weight loss, fever, or night sweats were noted in only 2 to 14% of patients in one series (Table 51-5), although, in a more recent series, fever and/or night sweats were present in 24%.<sup>73</sup> These symptoms are more common in patients whose disease is widespread than in those with more localized disease.<sup>73</sup> Pruritus,<sup>109</sup> a prominent symptom in patients with Hodgkin's disease (Chapter 50), is uncommon. Abdominal pain, nausea and vomiting, and dysphagia, resulting from gastrointestinal involvement or enlarged

Table 51-4. Relative Frequency of Various Histologic Types of NHL\*

Cell Type	Pattern	
	Nodular (% of patients)	Diffuse (% of patients)
Histiocytic	7.2	28.7
Mixed	18.3	10.5
Lymphocytic poorly differentiated (LLPD)	17.0	10.8
Lymphocytic well differentiated (LLWD)	1.5	2.5
Undifferentiated	0	3.5
Total	44	56

\*Values represent percent of 405 cases seen at Stanford Medical Center.<sup>75</sup>

Table 51-5. Symptoms and Signs at Diagnosis\*

	GFLSa	RCSa	LLSa
Number of cases	162	554	553
Symptoms			
Lymphadenopathy	74%	50%	57%
Fatigue or malaise	14%	12%	18%
Gastrointestinal symptoms	9%	25%	23%
Weight loss	2%	10%	9%
Fever or infection	6%	11%	13%
Bone pain	0%	7%	1%
Signs (most prominent site of involvement)			
Lymphadenopathy	79%	58%	65%
Enlargement of lymphoid tissue of naso-oro- pharynx	1%	7%	10%
Skin or scalp	2%	7%	4%
Mediastinal or hilar nodes	1%	3%	4%
Bone lesions	0%	8%	1%

\*Modified, from Rosenberg et al.<sup>109</sup>

retroperitoneal nodes, are unusual. Pain from bone lesions is uncommon except in children.<sup>113</sup> A variety of other complaints are encountered occasionally, reflecting the unusual forms of presentation (page 1584 and Chapter 54). For example, shifting pulmonary infiltration associated with eosinophilia (Loeffler's syndrome) has been the presenting feature.<sup>6a</sup> On the average, patients have been aware of enlarged lymph nodes for six months before consulting a physician.<sup>109</sup>

The manifestations and course of patients who, when first examined, have NHL that is apparently confined to nonlymphoid tissue are somewhat different and will be discussed in a later section (page 1584).

## Physical Findings

Enlarged lymph nodes (Fig. 51-4) are present in most patients with NHL and are especially common in those with GFLSa (Table 51-5). Cervical or supraclavicular nodes are most often involved. Fewer than 5% of patients have generalized adenopathy.

The nodes usually are nontender, freely movable, distinct, and rubbery, although in some patients with RCSa they are quite hard. Spontaneous fluctuations in the size of lymph nodes may be observed.<sup>6,129</sup> Tonsils and/or adenoids are visibly enlarged in about 10% of patients with LLSa, but are less commonly enlarged in those with GFLSa<sup>109</sup> (Fig. 51-5).

*Röntgenograms* of bone in those complaining of bone pain or tenderness usually disclose lesions (Chapter 54). Chest x ray revealed hilar or mediastinal nodes in a very small percentage of patients in one series (Table 51-5), but, in another series, evidence of mediastinal or hilar disease was found in 18% of patients with nodular lymphoma and in 24% of those with diffuse lymphoma.<sup>75</sup> Parenchymal lung involvement is rare at the time of diagnosis.<sup>109</sup> Retroperitoneal nodes may be palpable, but the spleen and/or liver are enlarged in only a small percentage of patients at the time of diagnosis.<sup>38,109</sup> *Splenomegaly* as the only sign of disease was noted in 2.5% of patients with GFLSa, but was present in fewer than 1% of those with LLSa or RCSa.<sup>109</sup> (See primary NHL of spleen, page 1585.)

Disease localized to a single lymph node region or to two contiguous lymph node regions (stage I disease) is unusual.<sup>2a,46</sup> In one series,<sup>45</sup> only 25% of patients had localized nodal disease detectable on physical examination, and, in a series of patients subjected to more extensive staging procedures, stage I or I<sub>E</sub> (extension to nonlymphoid tissue from a local involved node) was found in 12%.<sup>75</sup> In patients with stage I or II disease, lymphangiography revealed abdominal node involvement in 20%<sup>76a</sup> to 80%.<sup>83</sup> Local node disease may be most common in patients with GFLSa; in one study, 34 of 64 of these patients had disease apparently limited to one node group, but extensive staging procedures were not performed.<sup>56</sup> When NHL was classified as "diffuse" or "nodular" on the basis of any degree of nodularity constituting the latter, stage I was slightly more common in patients with "diffuse" (14%) than in those with "nodular" disease (11%).<sup>75</sup>



Fig 51-4 Extreme generalized adenopathy in a patient with "reticulum cell" sarcoma. The radiosensitivity of the tumor is indicated by the change produced in 13 days.

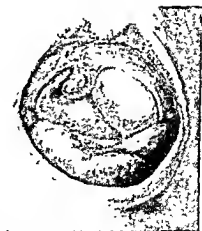


Fig 51-5 Nasopharyngeal tumor in a patient with follicular lymphoma. The uvula is displaced to the right.

Laboratory Findings<sup>20,59,109</sup>

Examination of the *blood* reveals normal values for blood cells in most patients at the time of diagnosis. Increased leukocyte counts are uncommon, as is leukopenia (Table 51-6). However, abnormal-appearing lymphocytes (page 1580) may be present in small numbers in a few patients<sup>20,109</sup> and these may lead one to suggest NHL as a cause of lymphadenopathy. Anemia is present in fewer than 50% of patients and is rarely severe at diagnosis, although Coombs'-positive hemolytic anemia may occur, especially in well-differentiated lymphocytic lymphoma (Chapter 54). The anemia usually is due to decreased production as evidenced by low reticulocyte numbers. Significant thrombocytopenia is unusual (Table 51-6).

In one study, *bone marrow* biopsy performed at the time of diagnosis revealed evidence of NHL involving the bone marrow in 18% of 356 patients.<sup>75</sup> In that series, marrow was involved somewhat more frequently in those with the nodular than in those having the diffuse varieties (22% vs 15%); marrow involvement was found in only 10% of patients with the histiocytic type, in 14% of those having the mixed type, and in 33% of those with the poorly differentiated lymphocytic variety. Of 12 patients with the well-differentiated lymphocytic type the bone marrow was involved in 42%. In another

study of marrow biopsy in 75 patients with well or poorly differentiated lymphocytic lymphoma, 63% had evidence of marrow invasion.<sup>137a</sup>

On biopsy in patients with marrow involvement, nodular accumulations of small lymphocytes are noted in those with the well-differentiated lymphocytic form and lymphoblasts and "reticulum" cells in those with the other varieties. In aspirates the percentage of lymphocytes may be increased in the former group or the finding of abnormal-appearing cells in the latter may allow one to make a diagnosis. Occasionally, in patients with anemia, often accompanied by leukopenia, neutropenia, and/or thrombocytopenia and no palpably enlarged lymph nodes or spleen, aspiration of bone marrow may reveal a marked increase in small lymphocytes. In such subjects, a diagnosis of well-differentiated lymphocytic lymphoma, apparently limited to bone marrow, must be considered but is difficult to establish without biopsy.

*Uric acid* may be increased in serum or urine, but more often is normal (Chapter 54). Hypercalcemia almost always is an indication of x-ray demonstrable destructive bone lesions.<sup>95</sup>

*Serum proteins* usually are normal when the diagnosis is made. However, hypoalbuminemia often develops as the disease advances and reduced levels of gamma globulin may be found, especially in patients with well-differentiated lymphocytic lymphoma, although with a lower frequency than in those with CLL<sup>22</sup> (Chapters 44, 54). Monoclonal paraprotein spikes without other evidence of myeloma or related diseases (Chapters 52 and 53) are seen occasionally.<sup>79</sup>

## Diagnosis and Differential Diagnosis

The diagnosis is made by observing the characteristic pathologic changes in biopsy material (page 1571), usually from excised lymph nodes but occasionally from other sites such as tonsils, bone marrow, spleen, liver, bowel, or skin.<sup>109</sup>

Table 51-6. Blood Values in Non-Hodgkin's Lymphoma at the Time of Diagnosis\*

	Percent of Patients
Hemoglobin	
Greater than 14 g/dl	40
12-13.9 g/dl	38
8-11.9 g/dl	20
Less than 8 g/dl	2
Leukopenia (less than $4.0 \times 10^9/l$ )	3
Platelets	
Greater than $200 \times 10^9/l$	60
100-200 $\times 10^9/l$	35
Less than $100 \times 10^9/l$	5

\* Adapted from Rosenberg et al.<sup>109</sup>

Difficulty in diagnosis is encountered in some 2 to 4% of patients who have no palpable evidence of disease and in whom the chest film shows no abnormality. Such patients may consult a physician because of fever, night sweats, or weight loss and in them the retroperitoneal nodes are often the site of disease.<sup>43,109</sup> When the primary finding is severe anemia, especially when accompanied by neutropenia and thrombocytopenia, bone marrow aspiration or, preferably, biopsy may disclose NHL. Such patients eventually may develop palpable lymphadenopathy or leukemic transformation may occur (page 1579), but others may die from infection or hemorrhage secondary to neutropenia and thrombocytopenia, respectively, while the NHL is still apparently limited to the marrow.<sup>92</sup>

There are numerous conditions, both benign and malignant, that are associated with symptoms, signs, and laboratory findings similar to those observed in NHL. The differential diagnosis depends upon the histologic features of NHL as distinguished from those of other causes of lymph node or lymphatic tissue enlargement or infiltration (Chapters 7 and 40). The demonstration of IgG on the cells in germinal centers may be helpful in distinguishing benign from malignant follicular hyperplasia.<sup>21a</sup> If examination of the blood discloses CLL, no additional diagnostic steps are necessary (Chapter 49). If blood lymphocytes are not increased, excisional biopsy of palpably enlarged lymph nodes is the next step to consider. In some patients, as mentioned above, bone marrow aspiration and/or biopsy may disclose marked lymphocytic infiltration or abnormal-appearing cells that suggest the diagnosis (see Chapter 40 for a more detailed discussion of diagnostic steps).

If a skilled pathologist considers the tissue to be clearly indicative of NHL, then, unless the patient has received diphenylhydantoin, related hydantoins (page 1553),<sup>62</sup> or perhaps other drugs, little doubt exists as to the diagnosis, for few or no other benign conditions mimic the pathologic manifestations of NHL. The opposite situation, in which an excised

node is considered nondiagnostic in a patient who eventually proves to have lymphoma, is much more common<sup>43</sup> and was discussed in Chapters 40 and 50. For differentiation between tropical splenomegaly and primary lymphoma of the spleen see pages 1411 and 1586.

As previously discussed (Chapter 49), no pathologic distinction between LLs and CLL has been demonstrated other than blood lymphocytosis in the latter.

## Course and Survival

Therapy (page 1581), while it may not always prolong life, nonetheless provides symptomatic relief and significantly modifies the expression of disease throughout its course. Thus, extreme enlargement of lymph nodes (Fig. 51-4) rarely is seen in treated patients. However, by the time death occurs, cervical, axillary, thoracic, and abdominal lymph nodes are involved in most patients with NHL and in approximately 50% of them the liver, spleen, and parenchyma of the lungs or kidneys are involved.<sup>109</sup> Spread of disease to virtually all organs can be observed at autopsy.<sup>59,103,109</sup>

### Causes of Death

An exact, identifiable single event often is lacking as an explanation for death. Many patients become increasingly debilitated with widespread lymphoma involving many sites and die without a clear-cut single cause. Death from bacterial infection is relatively common and death from fungal infection may occur (Chapter 54). In some patients the infection is attributable to deficient antibody production (Chapter 44), most commonly in the well-differentiated lymphocytic form of NHL (Chapter 54). In others, infection begins in tissue damaged by lymphomatous infiltration, eg, pneumonia secondary to parenchymal pulmonary disease. Since the tumor often is poorly responsive to chemotherapy as death approaches, attempts to control its growth with large doses of chemotherapeutic agents often induce neutropenia, contributing to the frequency of infection.

Neutropenia also may develop as a consequence of bone marrow invasion. Thrombocytopenia may have the same cause as neutropenia; death from thrombocytopenic hemorrhage is observed occasionally. In still other patients, death is attributable to organ failure secondary to lymphomatous infiltration of such tissues as lung, gut, kidney, or meninges (Chapter 54).

### Survival

Survival curves for NHL fit a log probability plot reasonably well.<sup>109</sup> This type of survival curve is common in many types of cancer, but does not describe survival well in other lymphoid neoplasms such as CLL (Chapter 49). Rosenberg et al<sup>109</sup> studied survival in 1269 patients whose lymphoma was classified by the method of Craver (Table 51-7). Of these patients, 50% survived longer than 26 months, and 28% survived for five years or more. Prognosis was much poorer in children than in adults and was slightly

better in females than in males. Those with disease apparently limited to one lymph node area lived significantly longer than those with more extensive disease. Patients with GFLSa lived approximately three times as long as those with LLSa or RCSa. Whether the better results in patients with disease that initially was localized reflected a high frequency of GFLSa was not determined. Gall and Mallory<sup>59</sup> reported longer survival in patients with well-differentiated GFLSa than in those whose follicles contained poorly differentiated cells, and an average survival for all patients with GFLSa of five years as compared to three years for those with LLSa and one or two years for patients with all other forms of disease. Others also found the median actuarial survival of GFLSa patients to be approximately five years<sup>20</sup>; the clinical extent of the disease was found to be more important than fine morphologic distinctions. In still other studies,<sup>86,87,104</sup> the prospects for survival also were best in GFLSa, intermediate in LLSa, and shortest in RCSa.

Survival, as determined in some series,<sup>59,86,87,101,109</sup> is probably underestimated by virtue of the statistical methods used in calculations. As discussed for CLL (Chapter 49), some forms of calculation by actuarial methods give a fairly accurate figure for expected survival. Such calculations for patients with NHL, treated during the past decade<sup>75</sup> (Fig. 51-6A and B), suggest the following median duration of survival: lymphocytic, poorly differentiated and mixed cellularity, diffuse varieties, two years; nodular varieties, seven to eight years; histiocytic, diffuse, one year; nodular, three to four years. The number of patients with well-differentiated lymphocytic<sup>16</sup> and undifferentiated<sup>14</sup> types was too small to derive a meaningful median survival, but the data suggested that the longest survival period was in the former group and the shortest in the latter.

Thus, of the various possible histologic types, nodularity appears to be the single most important factor influencing survival.

The above figures represent approximations of what may be expected of a large group of patients. However, the behavior of

**Table 51-7. Survival of Patients with Non-Hodgkin's Lymphoma at Memorial Hospital, Admitted 1928-1952 (1269 Patients)**

	Median Survival from Onset (Months)
All patients	26
Male	24
Female	32
Children	8
Type of disease <sup>a</sup>	
GFLSa	72
RCSa	25
LLSa	21
Clinical stage	
Localized	51
One side of diaphragm	22
Generalized	26
Year of admission	
1928-1934	27
1935-1939	29
1940-1944	28
1945-1949	23
1950-1952	27

<sup>a</sup>Onset of symptoms or from diagnosis in asymptomatic patients (Modified from Rosenberg et al<sup>109</sup>)



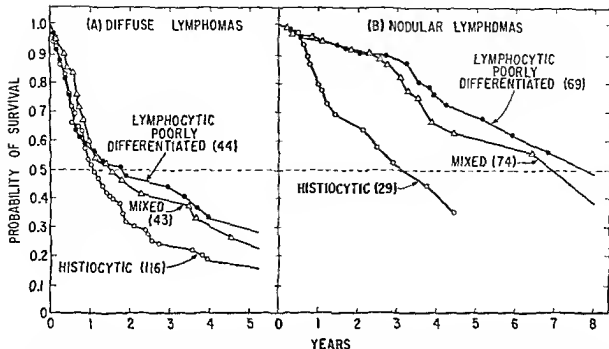


Fig 51-6 Actuarial survival of patients with non-Hodgkin's lymphoma treated at Stanford University, 1960-1971. The number in parentheses after each cell type indicates the number of patients in that group. Not shown are curves for the small number of patients with lymphocytic well-differentiated, diffuse (10), or nodular (B) lymphoma or with undifferentiated lymphoma (14). (From data of Jones et al.<sup>15</sup>)

these tumors varies greatly and survival is so different from one to another patient with an apparently similar tumor that these figures provide little help in estimating prognosis in individual cases.

In addition to age, sex, cell type, and apparent extent of disease (Table 51-7), other factors present at diagnosis may influence survival. Lymphopenia appeared to imply a grave prognosis in one series.<sup>109</sup> Median survival in patients with fewer than  $1.0 \times 10^9$  lymphocytes/l was three months; with  $1.0$ – $2.0 \times 10^9$ /l, nine months; and in those with more than  $2.0 \times 10^9$ /l, 18 months. In another series,<sup>75</sup> mediastinal involvement tended to connote a poor prognosis, but the presence or absence of fever and/or night sweats had no prognostic significance independent of that conferred by the extent of demonstrable disease. The influence of age varied according to cell type; no age effect was apparent in patients with diffuse histiocytic disease, young patients living longer than older patients with poorly differentiated lym-

phocytic or mixed cell types of the nodular variety, while the opposite was true in patients whose cell types were of the diffuse variety.<sup>75</sup> The type of detailed analysis of factors influencing survival that has been carried out in the leukemias (Chapters 47-49) and in Hodgkin's disease (Chapter 50) has not been made in NHL.

#### Leukemic Conversion of NHL (Lymphosarcoma Cell Leukemia, LSCL, Leukolymphosarcoma)

Overall survival from onset of NHL was not appreciably different for patients who did or did not develop leukemia, according to one study.<sup>109</sup> However, the prognostic implication of such a change depends on the nature of the leukemia. Thus, conversion to a picture of acute leukemia may occur and in this instance, prognosis is relatively poor. If a picture resembling CLL develops the outlook is much less grave.

The type of leukemia that develops is in-

fluenced by age and by the cell type of the original NHL. In patients with LL<sub>Sa</sub>, the lymphocytes in the blood may be indistinguishable from those of patients with CLL. In such patients it is only the knowledge of a preceding nonleukemic phase of NHL that allows one to make a diagnosis of LSCL rather than CLL. Patients with poorly differentiated lymphocytic lymphoma may develop LSCL with cells morphologically indistinguishable from the lymphoblasts of ALL. Again, in certain patients, the leukemic phase of NHL is associated with the appearance of cells that have morphologic features not commonly encountered in other forms of leukemia<sup>114,116</sup> (Fig. 51-7). Isaacs<sup>66</sup> was probably the first to draw attention to this condition. Most typically, these "lymphosarcoma" cells are large lymphocytes with large nuclei, but they possess more cytoplasm than

does the typical lymphoblast. The nuclear chromatin is clumped, and very large, often single, nucleoli are present. The nuclei are round or oval and may have deep clefts. The cytoplasm may stain lightly, deeply, or gray-blue with Romanowsky stains and may contain vacuoles, azurophilic granules, or, occasionally, basophilic-staining granules that appear to be structureless on electron microscopy (personal observation). Rarely, AML<sup>66</sup> or CML<sup>141</sup> may develop in patients with NHL, but whether this is a "conversion," the coincidental occurrence of two different diseases, or the result of therapy is unknown. The ultrastructure of well-differentiated and poorly differentiated lymphocytes in NHL is very similar to that of leukemic lymphocytes,<sup>17,128</sup> as is the fine structure of the nucleoli.<sup>124</sup> It has been suggested that lymphocytes from patients with

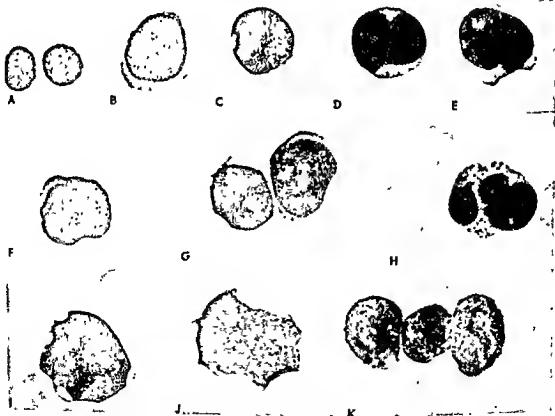
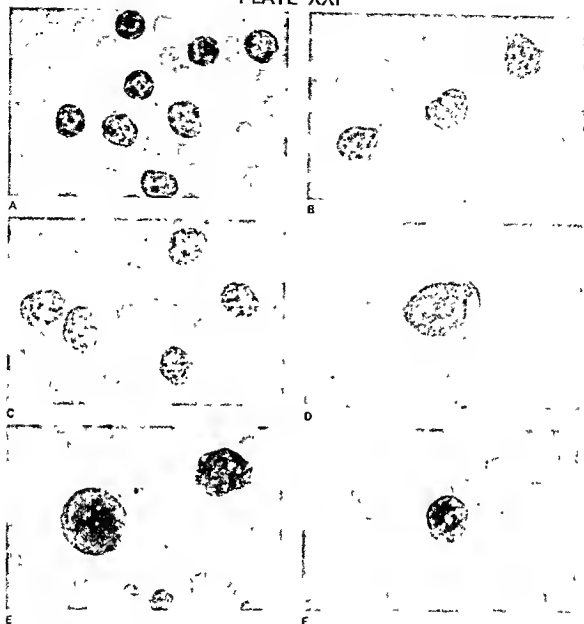


Fig 51-7. Leukocytes from lymphosarcoma cell leukemia. Small lymphocytes from a patient with CLL are shown in panel A for contrast with the larger and abnormal appearing "lymphosarcoma" cells in panels B through K. (From Schnitzer et al<sup>114</sup> courtesy of the authors and J B Lippincott Company)

# PLATE XXI



Cells from chronic lymphocytic leukemia and non-Hodgkin's lymphoma (Wright's stain,  $\times 1000$ ) A, B, Chronic lymphocytic leukemia C, Blood and D, E, bone marrow of three different patients with non Hodgkin's lymphoma, the course in patient shown in E was unusually rapid and resembled that of acute leukemia F shows the typical cells found in the blood in patient shown in E

lymphosarcoma may differ from those of patients with leukemia in propionate metabolism<sup>125</sup> and in cytolytic sensitivity to prednisone.<sup>116</sup>

Leukemic conversion of histiocytic lymphomas is uncommon.<sup>84,105,114</sup> When such conversion does occur, the cells invading the blood resemble those seen in the Schilling type of monocytic leukemia (Chapter 47), when examined by light or electron microscopy.<sup>114</sup>

Leukemic conversion of NHL in children is much more common than in adults, but conversion to a CLL-like picture is exceedingly rare in children.<sup>109</sup> Surveys suggest that one third to one half of children with NHL develop a leukemic phase.<sup>5,113</sup> This picture usually develops within a few weeks following the original diagnosis in children,<sup>74</sup> but may not develop for years in adults.<sup>109,114,117</sup> Rosenberg et al<sup>109</sup> found that 13% of children and 7% of adults developed a leukemic phase, but a later survey from the same institution disclosed a still higher frequency in children.<sup>23</sup> It may be inferred that both figures should be looked upon as minimal since cases in the early years of the series may not have been as well studied. In the series cited, leukemic conversion was noted in 13% of patients with LLs, in 9% with GFLs, and in only 2% with RCSs. Other studies bear out the infrequency of leukemic conversion in RCSs, especially the form characterized by well-differentiated histiocytes.<sup>105,106</sup>

Criteria for making a diagnosis of LSCL in a patient with NHL are somewhat arbitrary since a few peculiar-appearing cells can be observed in the blood of many patients. Persistent lymphocytosis exceeding  $5.0 \times 10^9/l$  is the usual finding on which such a diagnosis can be based, but various other criteria have been proposed.<sup>59,105,109</sup> If the blood is involved, the marrow is almost invariably infiltrated, but whether marrow infiltration without blood involvement should be considered leukemic conversion is debatable.

If the leukemic picture becomes one of CLL, little change may be noted in physical

signs or other laboratory values except that the spleen is more likely to be palpable than before such conversion occurred.<sup>109</sup> However, if the picture is one of acute leukemia, then anemia, neutropenia, and thrombocytopenia develop and often constitute the most important problems.

## Therapy

There are insufficient data in the literature to allow a rational choice of therapy in NHL.<sup>24,103a</sup> Radiotherapy to obviously involved areas,<sup>75,104</sup> total body x-irradiation,<sup>73</sup> administration of radioactive isotopes such as <sup>32</sup>P,<sup>47,50</sup> alkylating agents of all types,<sup>60,89</sup> antimetabolites such as methotrexate,<sup>102</sup> stathmokinetic agents such as vincristine and vinblastine,<sup>32,127</sup> procarbazine,<sup>126</sup> bleomycin,<sup>111</sup> streptonigrin,<sup>108</sup> and steroids used daily<sup>53</sup> or in intermittent dosage<sup>31</sup> have all been found useful. Each of these will reduce the amount of palpable or x-ray demonstrable disease in the majority of patients (partial remission) and lead to complete disappearance of apparent disease (complete remission) in some. Disease recurs in a short time in most patients, but may not recur for some years in a small percentage of patients. As discussed below, combining various useful therapeutic agents results in a greater proportion of patients achieving complete remission than do therapeutic modalities used singly, but this may be accomplished at the expense of increased morbidity during therapy as a result of drug toxicity.<sup>49</sup> Both "curative" and palliative therapy deserve consideration.

### "Curative" Therapy

As with Hodgkin's disease (Chapter 50), the patient should be evaluated with the object of attempting curative therapy. If the disease proves to be limited to one lymph node group, a rather rare event<sup>46,50,73</sup> (page 1575), or to one extranodal site, such as the stomach, radiotherapy probably should be considered the treatment of choice.<sup>75,103a</sup> Apparent cures have followed surgical exci-

sion of localized nodal or extranodal disease, but a nonrandom comparison of surgical versus x-ray therapy suggested that the latter was superior.<sup>109</sup> There is no evidence in NHL that the radical, extended field irradiation therapy recommended for Hodgkin's disease (Chapter 50) has any advantage over radiation therapy restricted to the involved areas.<sup>73,104</sup> However, data are presently inadequate to explicitly describe the best form of irradiation therapy for patients with localized NHL.<sup>56,71,72,98,104</sup> In terms of recurrence at an irradiated site, one report<sup>119</sup> suggested that RCSa and LLSa are similar to Hodgkin's disease in that recurrence is rare if 3500 rads have been delivered, but differ in that they recur more commonly if a smaller dose is used. Nevertheless, in patients with GFLSa, which is more radiosensitive, recurrences have been unusual when as little as 2000 rads were delivered.

The difficulty of defining cure of NHL is illustrated in the discussion of gastric (page 1584) or splenic non-Hodgkin's lymphoma (page 1585). Survival in those circumstances bears little relation to the type of therapy employed and relapse may occur many years after the original diagnosis and treatment. Cure of patients with Hodgkin's disease (Chapter 50) can be defined to some degree since results of therapy have been reported in terms of disease-free interval and survival has been compared to that expected in the general population. Similar analyses of results of therapy in NHL are rare,<sup>107a</sup> the usual reports referring to survival rather than disease-free interval.<sup>71,94,98,104</sup> (Fig. 51-6A and B). Since survival with NHL is highly variable, survival reports are difficult to interpret. Small series do indicate an appreciable frequency of disease-free, five-year survival in patients with GFLSa with localized nodal disease treated by excisional biopsy or by x ray in relatively low doses<sup>56</sup> as well as in patients with all types of stage I or II NHL who survived for five or more years following irradiation.<sup>137</sup> In one large series,<sup>73</sup> 215 patients staged as I to III<sub>E</sub> by the Ann Arbor staging classification for HD (Chapter 50), received initial therapy with radiation alone.

Duration of follow-up in the series was not long enough to allow firm guide lines to be drawn, but certain tentative conclusions were reached. In patients with diffuse lymphomas, histiocytic or lymphocytic, there was a very high rate of relapse in the first few years following either local or extended field irradiation. However, actuarial survival curves suggest that perhaps half of the small group of stage I and I<sub>E</sub> patients with diffuse disease may have been cured. In general, the configuration of the curves for patients with diffuse disease was similar to those seen in HD; the probability of relapse decreased very markedly with each succeeding disease-free year. Conversely, in patients with nodular disease there was little evidence for a reduction in the probability of relapse as the disease-free interval lengthened.

Comparable disease-free intervals have also followed chemotherapy<sup>112a</sup> or minimal radiotherapy and chemotherapy, and have occurred spontaneously.<sup>41,103a</sup> The entire concept of cure is made quite complex by the difficulty of distinguishing pathologically between benign lesions and well-differentiated NHL (page 1585). Most instances of localized, apparently cured disease involved such well-differentiated tumors that the "cured" disease may not have been lymphoma.<sup>50,105</sup>

### Palliative Therapy

If the patient is first seen when the disease is too extensive to consider "curative" therapy (page 1581), a period of observation may be advisable. Important considerations are the presence or absence of symptoms related to NHL, as well as the possibility of imminent complications of serious import, such as spinal cord compression (Chapter 54), and the degree of concern exhibited by the patient. The last factor is subject to considerable variation and is greatly influenced by the attitude of the physician and by his ability to convey to the patient a reasoned approach to the disease (Chapter 55). The advantage of a period of watchful waiting in patients who have relatively asymptomatic disease at the outset is that an opportunity is afforded

to assess the rapidity with which the disease is likely to progress without treatment. If rapid progression is observed, vigorous therapy may be in order, while, if little progression occurs, continued observation is justified. An example of the latter follows.

A 55 year old man was admitted to the hospital complaining of an enlarged cervical node, but he was otherwise asymptomatic. Biopsy of an enlarged node disclosed well-differentiated lymphocytic lymphoma, and bone marrow biopsy disclosed poorly organized nodules of lymphocytic infiltration. However, because the patient had no symptoms other than the enlarged node and his blood values were normal, no therapy was given. He chose to ignore the condition until six years after the diagnosis had been made, when he returned to "see how things were going." He was still healthy, still had palpably enlarged cervical nodes, and still had focal evidence of lymphoma on marrow biopsy even though his blood cell counts gave normal values.

In general it can be stated that follicular and lymphocytic lymphoma are more sensitive to therapy than is histiocytic. However, since individual patient response varies considerably, this general statement is of little use in specific instances. It has been said that bone marrow toxicity is no greater in NHL than in Hodgkin's disease with comparable doses of chemotherapeutic agents,<sup>81</sup> but our experience suggests that patients with NHL, particularly the lymphocytic forms, more easily develop pancytopenia during therapy. Thus, it seems reasonable to begin therapy with any marrow depressive agent at a lower dose in NHL than in HD until some assessment of tolerance has been obtained by observing the effects of the lower doses.

Palliative therapy (Chapter 55) is designed to reduce symptoms and to prolong comfortable life as well as to prolong life in the aggregate, if possible. Although unequivocal data from series of patients cannot be marshalled to provide evidence of prolongation of life as a result of therapy, there is little doubt that productive life has been increased in selected patients. When masses of lympho-

matous tissue produce local symptoms, their reduction by therapy decreases morbidity. Therapy of such complications as autoimmune hemolytic anemia can produce dramatic symptomatic relief (Chapter 54). Palliative therapy can be accomplished with x-irradiation<sup>107a</sup> or with chemotherapy. Combination chemotherapy, so useful in Hodgkin's disease (Chapter 50), has been employed in NHL, and has induced complete remission more often than when therapy with a single agent was given. However, the duration of response often has been short.<sup>84,85</sup> The frequency of complete remission following combined vincristine and prednisone appeared to be as high as when cyclophosphamide<sup>65,85</sup> or cyclophosphamide and procarbazine<sup>84</sup> were added to this combination. However, unmaintained remission may possibly be longer when vincristine, prednisone, cyclophosphamide, and procarbazine are used together<sup>84</sup> than when combinations of three or two drugs are used. It has been claimed that maintaining remission with various drugs may prolong the duration of remission in LLSa, but not necessarily in RCSa.<sup>85</sup> However, proof that maintenance therapy represents an advantage over reinduction of remission is lacking. Duration of survival of patients treated with combination chemotherapy was longer than in a group previously treated with sequential single-agent therapy,<sup>85</sup> but this was not a randomized comparison.

**THERAPY OF LEUKEMIC CONVERSION OF NHL.** If the conversion is to an ALL-like picture then the patient should be treated with the drugs used to induce and maintain remission in ALL (Chapter 47). Response to therapy may be as good in children developing ALL after a lymphosarcoma phase as in those having ALL initially,<sup>74</sup> but this has not always been true.<sup>5,128</sup> In general, the frequency of remission and the duration of remissions have been less in LSCL than in ALL.<sup>114,117</sup> Response to therapy of other forms of LSCL with alkylating agents has been disappointing in most cases.<sup>114,117</sup> Conversion to CLL should be approached in the

same manner as CLL (Chapter 49). Conversion to monocytic-histiocytic-reticuloendothelial cell leukemia is so infrequent that no useful therapeutic guidelines are available.

## Less Common Varieties of Non-Hodgkin's Lymphoma

Tissues other than lymph nodes may be the apparent primary site of origin of NHL, and since the clinical course of certain forms of *extranodal disease* may differ from that of disease beginning in lymph nodes, the extranodal type is discussed separately.

Lymphomatous tumors beginning in the skin will be discussed below in conjunction with two possibly distinct diseases, *mycosis fungoides* and *Sézary's syndrome* (page 1586). *Burkitt's (African) lymphoma* also is discussed separately (page 1590). Certain of the immunoglobulin-secreting tumors, especially those associated with *heavy-chain disease*, discussed in Chapter 53, have great clinical similarity to the non-Hodgkin's lymphomas. As noted in Chapter 49, *aleukemic chronic lymphocytic leukemia* is considered to be a form of LLs. Primary RCSa of bone is quite difficult to distinguish from *Ewing's sarcoma* on either histologic or clinical grounds<sup>60</sup> and consequently many authors exclude primary RCSa of bone from consideration with other lymphomas.<sup>109</sup> Malignant "histiocytoses" are hard to fit into a consistent classification of hematologic neoplasms, but as they must be distinguished from the lymphomas they are discussed in this chapter (page 1592).

### Extranodal Non-Hodgkin's Lymphoma

Initial clinical evidence of disease invading tissues other than lymph nodes or bone marrow is more common in NHL than in Hodgkin's disease, the lymphoid leukemias, or myelomas. In one large series,<sup>15</sup> 21% of the patients had evidence of invasion of tissues other than lymph nodes or bone marrow when first seen, and, in another series,<sup>109</sup> 37% had similar manifestations. It is unusual for follicular lymphoma to begin in this manner, but both the lymphocytic and histiocytic

forms do so. Unlike NHL arising in lymph nodes, extranodal disease is often localized to a single site.<sup>83</sup> Perhaps because of the tendency to remain localized, certain forms of extranodal disease offer a better chance for curative therapy than does primary lymph node disease.

The structures of the head and neck appear to be the most common sites. The tonsils, adenoids, and paranasal sinuses are most frequently involved, but the orbit, eyelids, tongue, thyroid gland, and virtually any other structure may be affected.<sup>45,109</sup> In one study of 292 patients with NHL which involved Waldeyer's ring, the disease was thought to be limited to that structure in 20%.<sup>74</sup>

*The gastrointestinal tract*, usually the stomach or less commonly small bowel or colon, is the initial site of disease in approximately 4% of patients with NHL and most cases are lymphocytic.<sup>45,76,109</sup> Gastric lesions may be ulcerative, diffusely infiltrative, polypoid, or appear as hyperplastic rugae or as combinations of these (Fig. 51-8). Initial symptoms are variable and include nausea, vomiting, anorexia, and vague abdominal pain as well as the classic symptoms of peptic ulceration. Protein-losing enteropathy may be found (Chapter 54).

Of 65 patients who proved to have gastric lymphoma and in whom no other evidence of lymphoma was present prior to surgical exploration, in 77% the disease was limited to the stomach; in the remainder the regional nodes were also involved.<sup>76</sup> However, gastric involvement is not necessarily the primary disease. In another series<sup>57</sup> of 75 patients in whom symptoms led to a diagnosis of gastric LLs in 64 and RCSa in 11, only 25 had disease apparently limited to the stomach. In still another group of 64 patients with involvement of stomach or bowel, all but two had disease elsewhere in addition to gastrointestinal disease.<sup>75</sup>

Prolonged survival and, in some instances, apparent cure are common in patients with lymphoma limited to the stomach. Survival of those with LLs, or in the rare instances in those with GFLs, was superior to that of patients with RCSa.<sup>76</sup> Similarly, patients



Fig. 51-8. Extensive involvement of the stomach such as may be seen in lymphosarcoma or in colloid carcinoma of that organ. The lesser curvature as well as the lower fundus and the pyloric regions are notably affected.

with small, superficial lesions appeared to have a better prognosis than those with the more extensive disease.<sup>76</sup> Although no comparative studies have been recorded, the apparent cure rate has been about the same whether the lesion was treated by excision, excision followed by radiotherapy, or biopsy followed by radiotherapy.<sup>57,76,109</sup> Survival of 59% of patients for five years and 28% for 10 years has been observed.<sup>76</sup> However, recurrence of disease as long as nine years after excision has been recorded.<sup>57</sup> The difficulty encountered in trying to relate survival or "cure" to the type of therapy employed is illustrated by a patient who remained apparently disease free for 15 years following partial excision of a gastric lesion even though he had enlarged regional lymph nodes and had not received radiotherapy.<sup>57</sup> The enigma of the lack of evident relation of type of therapy to end result has led to the suggestion

that certain gastric lesions considered to be lymphomas really represent benign lymphoid hyperplasia due to unknown cause.<sup>1,12,54,70</sup> It has been proposed that all lesions of the gut not associated with regional node involvement are "benign."<sup>1</sup> In a retrospective survey of pathologic material without knowledge of clinical outcome, when "benign lymphoid hyperplasia" was distinguished from "gastric lymphoma" on the grounds that benign disease is associated with long-standing peptic ulceration, particularly with overhanging ulcer margins, and the presence of other inflammatory cells and "true" germinal centers or extensive fibrosis,<sup>54,70</sup> only one of 21 patients classed as having "benign" disease was found to have died of lymphoma, while four of 13 classed as having lymphoma had done so.<sup>54</sup> This difference is suggestive but not statistically significant. In another series none of 12 considered to have benign disease had died while 14 of 15 with disease designated as malignant did so.<sup>70</sup> It is not clear, however, that this survey was made in a "blind" fashion. Thus, whether the unpredictable prognosis in gastric NHL is due to misdiagnosis of lymphoma or to innate variation in tumor behavior requires further study. Similar confusion exists concerning the less common primary involvement of the small bowel.<sup>103,139</sup>

*Primary NHL of the spleen* is in no sense common,<sup>2,20,42a,45,109</sup> but it presents two diagnostic problems. First, as with gastric lymphoma, it is possible that certain apparently cured primary lymphomas of the spleen (especially GFLSa) may have represented benign disease. Such cases include those reported as "cures" of primary GFLSa of the spleen following splenectomy, with survival for as long as 27 years.<sup>42,44,63,64</sup> Of 11 patients thought to have primary lymphosarcoma of the spleen at the time of splenectomy performed two to six years after a large spleen had been detected, six subsequently developed indications of CLL. The other five were alive without evidence of disease from one to 12 years after splenectomy even though focal lymphocytic infiltration had been noted in the liver at splenectomy.<sup>123</sup> In



a series of five patients in whom the spleen had a prominent follicular structure compatible with GFLSa at the time of splenectomy there was no evidence of recurrent disease at one, five, six, 10, and 13 years, postsplenectomy, respectively, despite the presence of increased lymphocytes in the bone marrow in all of them and blood lymphocytosis in some at the time of splenectomy.<sup>64</sup> Especially difficult to distinguish from primary NHL of the spleen on standard clinical grounds is "tropical" splenomegaly (Chapter 45). In the tropical disease, response to therapy with the antimalarial, proguanil, is expected; IgM levels are high and lymphocyte blastic transformation is normal; in contrast, IgM is normal or low and phytohemagglutinin (PHA) response usually has been abnormal in those proving to have NHL.<sup>112</sup>

Secondly, splenectomy is performed in occasional patients because of pancytopenia or a Coombs'-positive hemolytic anemia and yet histologic examination of the spleen fails to reveal a clear diagnosis. The spleen may have excessive numbers of small lymphocytes, but the distortion of architecture is so minimal that a diagnosis of LLSa cannot be made. Many of these patients later develop lymphoma, usually LLSa.<sup>10,64</sup> Dacie et al<sup>10</sup> reported nine patients with splenomegaly, all of whom were anemic and most of whom were neutropenic and thrombocytopenic but in whom no diagnosis could be made at the time of splenectomy. A diagnosis of LLSa was made in two of these patients, eight months and four years, respectively, after splenectomy; another died of unknown causes; six remained well with no diagnosis, two to seven years after splenectomy. Autoimmune hemolytic anemia has preceded the evident onset of RCSa by as long as seven years.<sup>38</sup> Whether in such cases lymphoma is present at the time of splenectomy but is unrecognizable by pathologic techniques, or whether the autoimmune phenomena precede and/or predispose to the development of lymphoma cannot be determined at present.

The diagnosis of lymphoma was made by diagnostic splenectomy in fewer than 1% of patients with lymphoma seen at the Mayo

Clinic<sup>2</sup> and in a series reported from India.<sup>45</sup> Of the 49 patients from the Mayo Clinic,<sup>2</sup> lymphoma was detected in abdominal lymph nodes or liver, as well as the spleen, in all but eight.

Primary NHL of bone most often is RCSa<sup>45,109</sup> although a variety of different histologic types may be encountered.<sup>69</sup> In view of the clinical differences between primary and secondary RCSa of bone and the histologic and clinical similarity of primary RCSa of bone to Ewing's sarcoma,<sup>50,109</sup> the "primary" condition may represent a different type of disease than other forms of NHL.

A variety of other sites have been noted as apparent primary sites of NHL, including skin, prostate gland, breast, kidney, bladder, ovary, testis, spinal cord, and various soft tissues.<sup>45,109</sup> In most cases, fatal dissemination has followed, although "cures" after excision or radiotherapy have occasionally been reported. Approximately 100 cases of NHL presenting as solitary pulmonary lesions have been reported. Such lesions may grow quite slowly, even if untreated.<sup>41</sup> However, the prognosis of seemingly localized extranodal NHL is not always better than that of nodal NHL; for instance, primary testicular disease has almost invariably been followed by generalized disease no matter what form of therapy has been employed.<sup>130</sup>

### Lymphomatous Tumors Beginning in the Skin

Any type of malignant lymphoma or leukemia may involve the skin as a manifestation of systemic spread of disease (Chapter 54) and, occasionally, NHL and Hodgkin's disease may begin as apparent primary tumors of the skin. In such instances, progression to a picture of generalized lymphoma usually, but not invariably, is observed.<sup>78</sup> There are, however, two primary tumors of skin, mycosis fungoides and the Sézary syndrome, that may be distinct from other lymphomas, although they may progress to diseases resembling systemic lymphoma and leukemia.<sup>19,51,105</sup> The term "mycosis fungoides" is an unfortunate one in that the disease is

not due to a fungus, as the name would imply. Furthermore, the term is not applied uniformly; some restrict its use to tumors with the "classic" histologic and clinical picture described below, while others apply it to any lymphomatous or leukemic process that begins in skin primarily.<sup>19,51,105</sup>

### **Mycosis Fungoides**

Mycosis fungoides may begin as a disease clinically and pathologically indistinguishable from psoriasis, seborrheic dermatitis, eczema, nonspecific exfoliative dermatitis, contact dermatitis, or neurodermatitis.<sup>18,51,105</sup> Pathologic distinction from these diseases becomes possible when the "indurative" or "plaque-forming" stage of the lesion develops. This, in turn, is followed by a "tumor-forming" stage.<sup>3,105</sup> The nonspecific skin eruption of the "premycotic" stage may persist for decades before the plaque-forming or tumor-forming stage is reached. In the largest series reported to date, the premycotic stage was present for a mean of four years.<sup>51</sup> Such a stage is not present in some patients; others seemingly go directly from the premycotic to the tumor-forming stage without an intervening stage of plaque formation.<sup>18</sup> Although rare in children, mycosis fungoides can occur in persons of any age and is slightly more common in males than in females.<sup>18,51</sup> The cells involved in the skin infiltrate may be histiocytes or plasma cells predominantly, or the cellular infiltration may be mixed and may include neutrophils and lymphocytes.<sup>3,105</sup> Epidermal changes are found in what is termed "mycosis fungoides,"<sup>45</sup> whereas primary skin involvement with lymphosarcoma or Hodgkin's disease usually does not include the epidermis. The epidermal changes consist of acanthosis, parakeratosis, elongated rete pegs, and spongiosis, in addition to "Darier-Pautrier abscesses," the intradermal clusters of histiocytes that often represent the first pathologic evidence of the presence of mycosis fungoides.<sup>105</sup> The indurative stage is characterized by cellular infiltration involving most of the upper dermis. As the tumor-forming stage is reached,

the infiltrate extends into the subcutaneous tissue.

The clinical appearance of the lesions parallels the pathologic evolution. A psoriatic-like plaque may be retained during the indurative stage and, as the tumor-forming stage is reached, the lesion becomes much more irregular. At all stages the skin feels indurated and firm. The absence of malignant characteristics in the cellular infiltrate in certain patients has led some to question the inclusion of mycosis fungoides with malignant neoplasms.<sup>3,105</sup>

At the time when a biopsy diagnosis is established, skin lesions clinically suggesting the premycotic stage are still found in approximately half the patients (Table 51-8). In one third, the tumor-forming stage has been reached by the time of diagnosis and, in these patients, ulceration and enlarged lymph nodes are fairly common (Table 51-8).

Anemia may be present and neutrophilic leukocytosis is frequent and appears to be correlated with the degree of skin infection.<sup>18</sup> Lymphocytosis may be detected, in which case some would consider the correct diagnosis to be Sézary's syndrome (see below). Eosinophilia may be noted. The bone marrow rarely is infiltrated but may contain increased plasma cells, perhaps due to the presence of chronic infection.

The tumor may be quite indolent; survival for many years is possible. Median survival from biopsy diagnosis in one series was four years and, when deaths apparently unrelated

**Table 51-8. Type of Skin Lesion and Frequency of Enlarged Nodes at the Time of Biopsy Diagnosis of Mycosis Fungoides (144 Patients)\***

	Percent of Patients
Skin tumors	33
Ulcerated tumors	13
Skin ulcers	15
Ulcers without skin tumors	2
Enlarged lymph nodes	28
No skin tumors, ulcers, or nodes	51

\*Adapted from Epstein et al.<sup>51</sup>

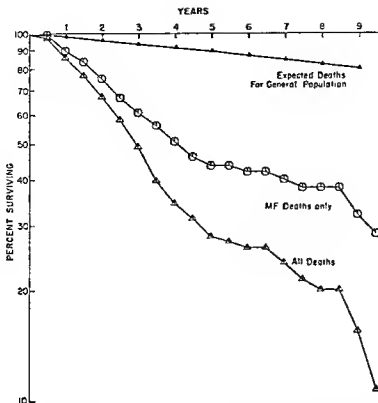


Fig 51-9 Survival from time of biopsy diagnosis of 144 patients with mycosis fungoides. Survival of all patients (lowest curve) those dying from mycosis (middle curve) and the expected age adjusted survival for the general population (upper curve) (From Epstein et al,<sup>51</sup> courtesy of the authors and Williams & Wilkins Company)

to mycosis fungoides were excluded, a "disease-oriented" median survival of five years was observed (Fig. 51-9).<sup>51</sup> A number of factors were found to influence survival.<sup>51</sup> Elderly patients survived a shorter time than those under the age of 50, even when non-tumor-related deaths were excluded. The presence of lymphadenopathy, skin tumors, and ulceration has prognostic significance (Fig. 51-10). When none of these was present, a median survival of eight years was observed, which declined to four years if one of these was present and to three years when two were present. A lymph node biopsy that discloses lymphoma is a poor prognostic sign as compared to one that does not provide a diagnosis. Duration of survival is quite short if hepatomegaly or splenomegaly is present.

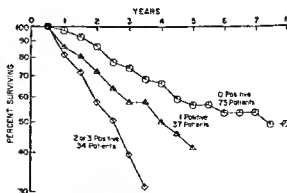


Fig 51-10 The influence of the presence or absence of cutaneous tumors, ulcers, or enlarged lymph nodes on survival in patients with mycosis fungoides. None of these abnormalities was present at the time of diagnosis in the patients shown in the upper curve, one of the three was present in those represented in the middle curve, and two or all three findings were present in those shown in the lowest curve (From Epstein et al,<sup>51</sup> courtesy of the authors and Williams & Wilkins Company)

Sex, race, and the duration of the premalignant stage had no prognostic implications.<sup>51</sup>

The majority of patients develop evidence of systemic lymphoma by the time of death<sup>51</sup>; however, in certain small series, systemic lymphoma developed only in a minority.<sup>3</sup> Biopsy of enlarged lymph nodes during life more commonly demonstrates reactive hyperplasia than lymphoma.<sup>3,51,105</sup> The type of lymphoma reported has been quite variable.<sup>3,18,51,105</sup> Hodgkin's disease, LLs, RCLs, and mixed lymphomas with cellular infiltration similar to that seen in the skin have been reported. The development of different cytologic types of lymphoma in the course of mycosis fungoides is one of the factors that has led some to suggest that mycosis fungoides is not a disease, per se, but rather a manifestation of any type of lymphoma beginning in the skin.<sup>107</sup>

Infection is the cause of death in most patients; this is usually spread terminally from chronically infected, ulcerative skin lesions.<sup>51</sup> As with other lymphomas, general debilitation and widespread disease may be present without an identifiable specific cause of death.<sup>51</sup>

### Sézary's Syndrome (Erythrodermia)

Sézary<sup>121</sup> described a series of patients<sup>120</sup> with a syndrome characterized by intensely pruritic generalized erythrodermia, atypical lymphocytic cells<sup>39</sup> in the blood, and cutaneous infiltration with atypical lymphocytes and histiocytes. Lymphadenopathy and hepatosplenomegaly often were present. The disease is rare, a total of about 30 to 40 patients having been reported in the medical literature.<sup>88,132</sup> Prolonged survival is the rule, patients living an average of approximately five years from onset of symptoms.<sup>26</sup> An identical clinical picture can be observed in patients with a CLL-like disease (Fig. 51-11), in those who initially have lymphocytic skin infiltration and eventually develop CLL, or in patients in whom the findings on skin biopsy are interpreted as indicative of mycosis fungoides.<sup>51</sup> Thus, to distinguish this syndrome from CLL and mycosis fungoides,



**Fig 51-11** Leukemia cutis universalis. The clinical examination of this patient revealed findings very similar to those designated as the Sézary syndrome, yet examination of his blood suggested a diagnosis of chronic lymphocytic leukemia. There was desquamation of the skin of the whole body and most of the hair was gone. The skin of the face, forehead and ears was swollen and the eyelids were infiltrated. The leukocyte count was  $30 \times 10^9/l$ , of which 73% were lymphocytes. All the accessible lymph nodes were greatly enlarged. On section, the cutis was found to be densely infiltrated with lymphocytes. The patient died of lobular pneumonia. None of the lymph nodes in the interior of the body was enlarged. The spleen was not increased in size, and there was only slight periportal infiltration of lymphocytes in the liver.

it is necessary to identify the "characteristic" blood cell or cellular infiltration of the skin. In one patient with Sézary's syndrome, the lymphocytes responded to PHA and showed chromosome abnormalities,<sup>39</sup> unlike the lymphocytes in CLL (Chapter 49). The similarity of appearance on electromicroscopy of "Sézary cells" and the cells infiltrating the skin in mycosis fungoides has led to the suggestion that the Sézary syndrome may be the leukemic phase of mycosis fungoides.<sup>88</sup>

The cells in the blood usually are numer-

ous enough to produce leukocytosis. They are rather large, 15 to 25  $\mu$ m in diameter, with irregular cytoplasmic borders in stained, fixed blood smears. The cytoplasm is light to dark blue and is fairly abundant. The cells often stain intensely with PAS (page 29), presumably reflecting a high glycogen content. However, intense staining of lymphocytes with PAS also may be observed in CLL and in LSA or in nonmalignant diseases such as infectious mononucleosis.<sup>93</sup> The suggestion that the Sézary cell could be distinguished by diastase-resistant PAS positivity<sup>131</sup> was not confirmed.<sup>31</sup> The nuclei are oval or round or may have clefts, and the nuclear chromatin is densely clumped. Nucleoli usually are not apparent in Wright-stained smears. The membrane characteristics of Sézary cells are those of "T" lymphocytes,<sup>26a,146</sup> while the cells in most lymphomas and CLL have the characteristics of "B" lymphocytes (Chapter 46). That these cells can be distinguished morphologically from the atypical lymphocytes seen in the blood of some patients with otherwise clinically typical CLL (Chapter 49) or lymphosarcoma invading the blood (page 1579) has not been demonstrated in a controlled study.

Pruritus almost always is the initial complaint. The skin appears red and is diffusely thickened. Demarcation between involved and uninvolved areas is poorly defined. The infiltration may involve the entire skin and in its extreme form often results in extensive exfoliation (Fig. 51-11). Edema of patchy nature often is a feature, and hyperpigmentation develops commonly. Body hair is lost and, with scalp involvement, total alopecia may develop. Hyperkeratosis of the palms of the hands and soles of the feet is common and fingernails and toenails become dystrophic and may be lost as nail beds are affected.

The disease may remain localized to the skin for some years but, in most subjects, lymphadenopathy and hepatosplenomegaly occur eventually. The cells infiltrating lymph nodes and other organs are similar to the cells in the blood.

### Therapy of Mycosis Fungoides<sup>51,91,136</sup> and Sézary's Syndrome<sup>30,34</sup>

Systemic chemotherapy may have a prolonged palliative effect, corresponding to what is expected with other forms of lymphoma (page 1581). Alkylating agents, such as chlorambucil and cyclophosphamide, HN2, and BCNU, and antimetabolites, such as methotrexate, often are beneficial.<sup>51</sup> Prednisone may produce dramatic effects in erythrodermia and in other forms of skin involvement with lymphocytic cells, but often is less effective in "classic" mycosis fungoides. Azaribine, a purine analog, has been claimed to be one of the most effective of the therapeutic agents.<sup>91</sup>

Irradiation of the skin with rays of low penetrance, such as electron beams, often is helpful, but the machines delivering such rays are available only in a few specialized treatment centers.<sup>51</sup> Periodic application of 10 to 40 mg of nitrogen mustard, dissolved in 30 to 60 ml of tap water, to the entire skin surface has been found useful in patients with the diffuse type of skin lesions but has not been as effective when discrete tumors were present.<sup>136</sup> General application of 10 mg HN2 (dissolved in 60 ml of water) to all of the skin two to three times each week, coupled with local injection of nodular lesions, led to complete remission, often of long duration, in 50% of 75 patients so treated.<sup>136a</sup> Although hypersensitivity to HN2 was a frequent complication, desensitization with small, intravenous doses proved feasible. The completeness of the response to the first course of therapy bears little, if any, relationship to duration of survival.<sup>51</sup> Therapy, although palliative, may not prolong life.

### Burkitt's Lymphoma (African Lymphoma)

A form of sarcoma that is predominantly extranodal and has a special and unique predilection for the jaw and facial bones was described in natives of Uganda by Burkitt.<sup>1,28</sup> The disease is common in certain parts of Africa and New Guinea, but is rare in other

portions of the world. As discussed in Chapter 46, the evidence for an etiologic relation of Burkitt's lymphoma to a virus is perhaps stronger than for any other form of leukemia or lymphoma. The relatively recent recognition of this tumor probably reflects the lack of consistent medical care in most parts of Africa, rather than the development of a new disease; the clinical syndrome apparently was known many years ago.<sup>99</sup>

The tumor usually, but not exclusively, is found in children.<sup>99,145</sup> Males are affected slightly more often than females. The tumor is extranodal in most patients at the time of diagnosis, but lymph nodes, liver, and spleen may be involved when the diagnosis is made or at autopsy. In the majority of patients, more than one site is affected at the time of diagnosis. When the tumor is confined to a single site, it most often is found in the facial bones, especially in the mandible and maxilla (Fig. 51-12). Abdominal and pelvic viscera, retroperitoneal soft tissues, salivary glands, thyroid gland, long bones, and the central nervous system are sites involved with some frequency. Unlike other types of NHL in children, leukemic conversion is rare, at least in Africa.<sup>37</sup> Approximately 16% of these patients develop tumor in marrow, but even in these the blood usually is spared.<sup>21</sup>

The tumor consists primarily of large, immature lymphoid cells interspersed with isolated large macrophages, thereby producing its characteristic "starry-sky" histologic appearance (Fig. 51-13).

Burkitt's tumor is remarkably sensitive to chemotherapy with cyclophosphamide or methotrexate.<sup>102</sup> More than half the patients have achieved a complete remission after a single dose of cyclophosphamide, 40 mg/kg,<sup>33</sup> and approximately 20 to 50% were apparently cured by this method.<sup>29,36,140</sup> Patients who fail to achieve remission with a single dose usually do so after repeated doses. Long-term, disease-free interval following therapy is more common if the disease is localized rather than disseminated, but dissemination does not preclude a good prognosis.<sup>138a</sup> The same percentage of patients achieving re-



Fig 51-12 Burkitt's lymphoma involving mandible, maxilla and orbit (From O Conor,<sup>99</sup> courtesy of the author and Cancer Research)

mission and long-term survival was observed when remission was induced with six doses of cyclophosphamide as when induced with combination chemotherapy employing cyclophosphamide, vincristine, methotrexate, and cytosine arabinoside.<sup>144</sup> In a study in which various forms of therapy were used, 80% of 130 patients eventually achieved remission.<sup>145</sup> Spontaneous remission also may occur.<sup>30</sup>

A tumor with apparently identical morphologic appearance is seen occasionally in American children<sup>33,49,101</sup> or in children in other countries such as Colombia<sup>10</sup> or India.<sup>45</sup> In these children, abdominal tumors are more common, tumor of the facial bones is less common, and bone marrow involvement is more frequent than in African children. African children with the tumor tend to have increased serum IgG levels and decreased IgM, whereas in American children IgG has been normal or decreased and IgM

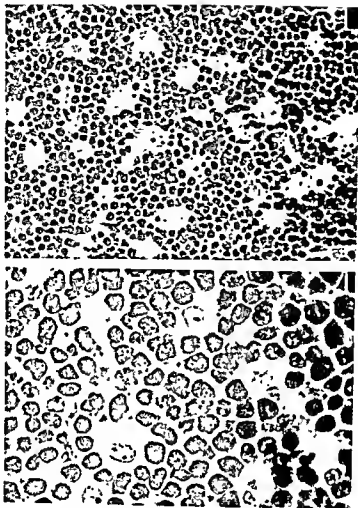


Fig 51.13 Histologic appearance of Burkitt's lymphoma. There is a uniform infiltration with immature lymphocytes, among which histiocytes are interspersed giving the so-called "starry-sky" appearance (Magnification  $\times 400$  and  $\times 1000$ , respectively) (From O'Connor,<sup>100</sup> courtesy of the author and American Journal of Medicine)

normal. A small group of American children have experienced prolonged remissions following cyclophosphamide therapy similar to those observed in Africa. Whether the non-African disease is the same as or different from the African variety must await further study

### Reticuloendothelioses

In 1923, Ewald<sup>12</sup> described a disease that he termed "leukemic reticuloendotheliosis." The two characteristics that he considered

distinctive were a very large spleen and increased mononuclear cells with numerous cytoplasmic projections in stained smears of blood. A variety of reports of diseases that bear some similarity to that described by Ewald have appeared under titles such as "histiocytic medullary reticulosis,"<sup>117a</sup> "familial haemophagic reticulosis,"<sup>55</sup> "reticulosis,"<sup>55</sup> "aleukemic reticuloendotheliosis,"<sup>58</sup> "reticuloendotheliosis,"<sup>96</sup> "lymphoreticular neoplastic disease,"<sup>115</sup> "reticulum cell leukemia,"<sup>81,132</sup> "chronic reticulolymphocytic leukemia,"<sup>110,131</sup> and still other terms.<sup>105</sup> These

are thought to be neoplastic diseases of the reticuloendothelial system. Their identification as distinct syndromes is as difficult as defining the reticuloendothelial system (Chapter 8). The relationship of some of them to acute or chronic monocytic leukemia (Chapters 47 and 48) or to RCSa or leukemic conversion of NHL is uncertain.

From information in the literature and from limited personal experience with such patients, for they are rare, it seems justifiable to recognize certain reticuloendothelioses as malignant, neoplastic diseases that can be separated from other forms of leukemia or lymphoma on the basis of histologic findings and perhaps on clinical grounds. Most case reports, no matter what term was employed by the authors, would appear to fit one of the following described syndromes: leukemic or aleukemic reticuloendotheliosis, histiocytic medullary reticulosis, and familial haemophagic reticulosis. Furthermore all of these have certain features in common and whether they are different diseases or variants of the same disease cannot be determined at present.

#### Leukemic and Aleukemic Reticuloendotheliosis

The clinical features<sup>4,31,55,58,110,115,133,135,142</sup> can be summarized as follows. Most patients are elderly, although persons of virtually any age may be affected; males are more commonly affected than females. Presenting complaints are fatigue, malaise, infection, and/or abdominal discomfort. The spleen is enlarged in most patients, often filling the left side of the abdomen. Hepatomegaly is common and lymphadenopathy often has been described although the nodes have been small in most patients. Fever, without evident infection, is common. Mild to moderate anemia is detected in most patients, as is moderate to severe thrombocytopenia. Neutropenia often is present but the total leukocyte count is low, normal, or elevated, depending upon the number of abnormal mononuclear cells in the blood. If significant numbers of abnormal cells are found in the blood, the disease is said to

be "leukemic," if absent, "aleukemic," but other differences between such patients are not apparent.<sup>58</sup> Eosinophilia may occur.<sup>82</sup> Marrow aspiration may reveal the abnormal-appearing cells or a "dry tap" may be obtained, in which instance the marrow biopsy may disclose a hypoplastic parenchyma with the abnormal cells present in moderate numbers.

The diagnosis depends on the histologic appearance of the infiltration, whether in blood, spleen, marrow, or lymph nodes. In sections of tissue, the infiltration is distinguished from the histiocytic infiltration in certain forms of lymphoma by being diffuse in nature rather than occurring in nodular aggregates. Furthermore, if spleen or lymph node cells are examined in a living state after having been teased free from tissue, they show the characteristic flagellated appearance that characterizes the cells described in the blood.<sup>115,133</sup>

The abnormal cells in the blood have been referred to as "hairy" cells because of the irregular cytoplasmic villi that give the cell a flagellated appearance in stained (Fig. 51-14A), living (Fig. 51-14B), or electron microscopic preparations.<sup>115,133,142</sup> The cell is large, usually 15 to 30  $\mu$ m in diameter, with a round or oval nucleus and fairly abundant gray-blue cytoplasm. The nuclear chromatin is moderately clumped and nucleoli usually are not visible or are small. Because its cell line of origin is unclear,<sup>115,133</sup> such terms as "chronic reticulolymphocytic leukemia"<sup>110</sup> have been used. Histochemical and cellular function studies suggest that the "hairy" cell shares certain characteristics common to both lymphocytes and monocytes. Acid phosphatase activity in the cells of patients with leukemic reticuloendotheliosis is prominent and is resistant to degradation by tartrate, whereas the cells in those with CLL or LSA have minimal activity and this is degraded by tartrate.<sup>143</sup> Other studies have suggested that the cell is lymphocytic.<sup>31a</sup>

The disease characteristically follows a rapid course with death usually occurring within four to six months after the diagnosis has been made. No form of therapy is of





Fig 51-14 "Hairy" cells from an imprint of spleen (A) and from a buffy coat preparation (B) from a patient with leukemic reticuloendotheliosis. (From Trubowitz et al.<sup>133</sup> courtesy of the authors and Grune & Stratton.)

predictable benefit, although splenectomy has been reported to lead to improvement in some patients.<sup>33a</sup>

### Histiocytic Medullary Reticulosis

Histiocytic medullary reticulosis was first described in 1939 by Scott and Robb-Smith.<sup>117a</sup> From clinical<sup>88</sup> and pathologic<sup>89</sup> study it is difficult to distinguish this syndrome from "aleukemic reticuloendotheliosis"<sup>58</sup> except for two features, namely, (1) prominent erythrophagocytosis by histiocytes

and (2) anemia due, at least in part, to a hemolytic process. The hemolysis is thought to be due to erythrophagocytosis by the histiocytes.<sup>97</sup> More than 50 cases have been reported,<sup>34</sup> but some of these patients have not exhibited erythrophagocytosis and how such cases differ from cases of "aleukemic reticuloendotheliosis" is uncertain.<sup>58</sup> The clinical features of histiocytic medullary reticulosis<sup>34,39,88,120</sup> are those described above for reticuloendotheliosis. The initial symptoms are fever, fatigue, weight loss, and/or an abdominal mass. The physical findings

consist of splenomegaly, often with hepatomegaly and lymphadenopathy, and, most commonly, pancytopenia. Fatal hemorrhage associated with thrombocytopenia and evidence for massive platelet phagocytosis by histiocytes has been reported.<sup>118</sup> A histiocytic leukemic phase has not been described, but a blood picture resembling ALL (Chapter 47) has been found at the initial examination.<sup>34</sup> Inclusions in erythrophagocytic histiocytes have been thought to resemble the cells seen in Niemann-Pick disease (Chapter 42), but the lipid profile was not compatible with the latter disease.<sup>138</sup> The course is rapid, survival for more than six months being unusual, but a few patients have improved after splenic irradiation or splenectomy,<sup>115</sup> or with steroid therapy.<sup>34</sup>

*Familial hemophagic reticulosis*<sup>9,55</sup> apparently is indistinguishable from histiocytic medullary reticulosis except for its familial occurrence. Four infant siblings were affected in one family in which both parents were healthy.<sup>55</sup> Consequently, the disease may represent an autosomal recessive genetic defect.

The reliability of the single factor, presence or absence of erythrophagocytosis by histiocytes, as a means of distinguishing between two forms of a disease can be questioned. Phagocytic capacity often is considered a function acquired by such cells as they mature (Chapters 6 and 45). It seems possible that all of the "reticuloses" represent one basic disease and that histiocytic medullary reticulosis represents a more differentiated form of disease than aleukemic or leukemic reticuloendotheliosis. In addition to differentiation from other leukemias and lymphomas, these reticuloendothelioses must be distinguished from other idiopathic diseases characterized by excessive numbers of histiocytes in the tissues. The clinical manifestations of histiocytosis-X (eosinophilic granuloma, Hand-Schüller-Christian disease, and Letterer-Siwe's disease) (Chapter 42) differ from those of the "reticuloses" and, in addition, in histiocytosis-X the histiocytes stain with Gomori's silver-impregnation method, while those of the reticuloendothelioses do not.<sup>48</sup>

*Multicentric reticulohistiocytosis*, while similar in title to the malignant reticuloendothelioses, is a crippling, but not fatal, disease associated with histiocytic infiltration of joints and skin that produces chronic deforming arthritis.<sup>8</sup>

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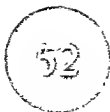
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## Plasma Cell Dyscrasias. Multiple Myeloma

### General Considerations

Definition

History

Etiology

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### Multiple Myeloma

Definition

Incidence

Clinical Manifestations

Laboratory Manifestations

Diagnosis

Treatment

Therapy of Complications

Prognosis

## General Considerations

### Definition

The plasma cell and lymphocyte dyscrasias include a number of disorders that share two basic characteristics: (1) the seemingly uncontrolled proliferation of cells normally involved in antibody production; and (2) the synthesis and secretion by these cells of a structurally homogeneous gamma globulin ("M-component") and/or its constituent polypeptide subunits. The structure of these proteins and the function of their "normal" immunoglobulin counterparts were discussed in Chapter 7.

These dyscrasias include (1) multiple myeloma, which is the most common; (2) Waldenström's macroglobulinemia; (3) the

heavy chain diseases; (4) "benign" monoclonal hypergammaglobulinemia; (5) certain forms of "essential" monoclonal cryoglobulinemia; and, probably, (6) amyloidosis. On the basis of morphologic and clinical criteria, Waldenström's macroglobulinemia and heavy chain disease are more properly identified as lymphocyte dyscrasias, whereas the others are plasma cell dyscrasias, but the latter term is usually used in referring to all these disorders.

### History

The early development of our knowledge of multiple myeloma<sup>40,83,141,261</sup> was described in an earlier chapter (page 1433). Since then the fundamental studies of Grabar, Heremans, Waldenström, Osserman, Putnam, Edelman, Franklin, and others have further delineated the clinical, pathologic, and immunochemical characteristics of these diseases (see below).

### Etiology

Some of the more general aspects of the pathogenesis and etiology of the neoplastic diseases of the hematopoietic system were discussed in Chapter 46. Here the plasma cell dyscrasias will be considered more specifically.

The exact cause of plasma cell dyscrasias remains obscure, but possible clues have

come from experimental studies in animals<sup>187</sup> and from certain clinical observations in man. The importance of *genetic factors* is suggested by the observation that spontaneously occurring plasma cell dyscrasias in mice,<sup>200,201</sup> those occurring in association with cecal ulceration,<sup>63,182,191</sup> and those induced by mineral-oil injection and similar means<sup>185,188,190</sup> are confined to specific strains of mice. In man a role for genetic factors is suggested by reports of plasma and lymphocyte dyscrasias occurring in siblings and other near relatives.<sup>4,15,33a,97,162</sup>

*Chronic stimulation of the reticuloendothelial system* also may be an important factor in the development of plasma cell dyscrasias. Thus the development of plasma cell tumors in anatomic proximity to chronic cecal irritation in C3H mice<sup>63,182</sup> and the development of myeloma following injection of Freund's adjuvants, mineral oil, and plastics in BALB/c mice<sup>185,188,190</sup> have been quoted in support of this view. In man, plasma cell dyscrasias of all types have been found in conditions associated with chronic RES stimulation, such as chronic osteomyelitis, pyelonephritis, tuberculosis, and chronic hepatitis.<sup>173</sup> The association between chronic infection and amyloidosis also is well known.<sup>174</sup> In addition, the association of human plasma cell and lymphocyte dyscrasias with rheumatoid arthritis,<sup>80,256,268</sup> Sjogren's syndrome,<sup>265</sup> and other autoimmune diseases is noteworthy. It is possible, of course, that a third independent factor may be responsible for both sets of clinical manifestations.

More recently, *viruses* have been suspected as etiologic agents in plasma cell dyscrasias. Thus, Aleutian mink disease, which is caused by a transmissible viral agent, is characterized by various manifestations of chronic viral infections such as lymphoreticular hyperplasia, autoimmune phenomena, and hypergammaglobulinemia.<sup>183</sup> About 10% of the infected animals also develop a monoclonal gamma globulin pattern and some excrete Bence Jones protein. Again, in mice, intracisternal A particles have been found in every plasmacytoma so far examined by electron microscopy<sup>54</sup> and, while it has been

claimed that these particles may simply represent evidence for viral superinfection in immunologically debilitated hosts, it is equally possible that they represent defective tumor viruses. The association of an unusual type of RNA-dependent DNA polymerase enzyme with intracisternal A particles strengthens the latter argument.<sup>260</sup> Furthermore, in addition to A particles, two types of C-type particles have been described in association with mouse myeloma cells. One contains the antigens associated with MuLV (Gross) virus infection,<sup>112,255</sup> whereas the other is a new virion antigen, VEA,<sup>12</sup> which is found on the surface of some mouse plasmacytomas (BALB/c) but not others. It was also shown that normal BALB/c mice have natural antibodies against this antigen.<sup>90</sup> Finally, myeloma has been transmitted from man to immunoincompetent animal hosts by intact myeloma cells or unirradiated cell filtrates,<sup>158</sup> suggesting a role for a filtrable but viable agent in the transmission of myelomatosis.

### The Protein Abnormalities

Characteristic protein abnormalities are central features of plasma cell and lymphocyte dyscrasias. The homogeneity of these proteins has made them invaluable tools in the hands of immunochemists and is in large measure responsible for our understanding of normal immunoglobulin structure and function. In addition, these proteins are of considerable importance in the diagnosis of multiple myeloma and related disorders, and sometimes their special properties contribute directly or indirectly to the clinical manifestations of these diseases. It is therefore fitting that their structural and functional characteristics be considered before discussing the clinical manifestations of the various plasma cell dyscrasias. Readers unfamiliar with the nomenclature and structural properties of immunoglobulins are referred to Chapter 7 (pages 305 to 313); tests for the identification and measurement of specific normal or abnormal immunoglobulins are discussed on pages 337 to 339.

Increased concentrations of structurally homogeneous proteins, called "M" (Myeloma or Macroglobulinemia) components, are found in the serum and/or urine of most patients with plasma cell dyscrasias. Such M-components occur in three major patterns: (1) complete gamma globulin molecules which may be IgG, IgA, IgM, IgD, or IgE, and may contain either  $\kappa$  or  $\lambda$  light chains; (2) free  $\kappa$  or  $\lambda$  light chains, either alone or in addition to complete immunoglobulin molecules carrying the same light chain type; and (3) fragments of heavy chain only, generally without the concomitant production of free light chains. When examined by *electrophoretic techniques* (page 337) these proteins appear as tall, narrow, sharply defined peaks that reflect their structural homogeneity (Fig. 52-1). For diagnostic purposes these characteristic contours are of greater importance than the height of an individual spike, although the latter is a good index of the amount of protein present. Electrophoresis also indicates the relative mobility of a given protein ( $\alpha$ ,  $\beta$ ,  $\gamma$ ) as well as the distribution and concentration of the normal serum proteins. Thus IgG has  $\gamma$  mobility predominantly, IgA has  $\beta$  mobility, and IgM, IgD, and IgE have  $\gamma$  to  $\beta$  mobility (Chapter 7).

The structural identity of M-components is usually established by *immunoelectrophoretic techniques* (page 338). First, polyvalent antisera containing antibodies against all serum components are used (Fig. 52-1). In such a test, normal serum proteins are recognized by the characteristic patterns and positions of their arcs; M-components, while maintaining their appropriate electrophoretic position, appear as thickened arcs with smaller than usual radii. These characteristics reflect their greater structural homogeneity and compact pattern of mobility. In addition to complete M proteins, immunoelectrophoresis may also detect gamma globulin fragments; free light chains usually migrate with  $\gamma$  to  $\alpha_2$  mobility, whereas free heavy chain fragments are generally found in the  $\gamma$  or  $\beta$  regions. In contrast to plasma cell dyscrasias, conditions associated with diffuse hypergammaglobulinemia, such as chronic

infections, show thickened immun arcs that have maintained their no. tours (Fig. 52-1).

While immunoelectrophoresis w valent antisera usually is conclus laboratories prefer to establish the specificities of a given M-compone use of univalent antisera with spec one of the five identifiable H chau of the two known L chains. In addit ous techniques have been develo make it possible to determine the ex tity of a given protein in the serum (Chapter 7).

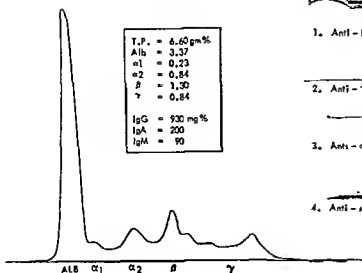
The presence of *light chains* in is best detected by electrophoretic te that typically reveal the presenc M-component, with  $\gamma$  to  $\alpha_2$  mobil concentration greater than that of The findings may be confirmed by i electrophoretic techniques using specific for  $\kappa$  or  $\lambda$  chains. Wheo ligl have the thermal properties of a *Be. protein*, their presence can also be o by precipitation at 50° to 60° C dissolving at 90° to 100° C. The reappears on cooling. The incidence tive reactions is maximal if the pE urine is carefully adjusted to 4.5 to ! times, but even under optimal cc some light chains do not give the teristic thermal pattern.

If the common form of proteinuria ent, confusion may arise since other will also precipitate at higher temp Such proteins must be filtered off af have coagulated at boiling and the Jones proteins are redissolved. The then repeated. Alternatively, the *tol. fonic acid (TSA) test*<sup>43</sup> can be used situations. In this test, 1 ml of TSA (12 g para-toluene sulfonic acid in s glacial acetic acid to make 100 ml) i to 2 ml of fresh urine by allowing the to run slowly down the side of the n tube is flicked with the finger and if t tion is positive, a precipitate will within 5 minutes. Albumin in concen up to 25 g/dl or  $\alpha$ ,  $\beta$ , and  $\gamma$  globulir 500 mg/dl will not interfere with th

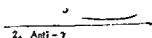


A

## NORMAL HUMAN SERUM

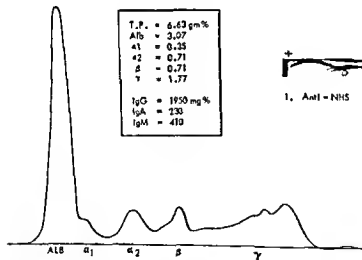


1. AntI - NHS

2. AntI -  $\gamma$ 3. Ants -  $\alpha$ 4. Anti -  $\mu$ 

B

## HYPERGAMMAGLOBULINEMIA



1. AntI - NHS



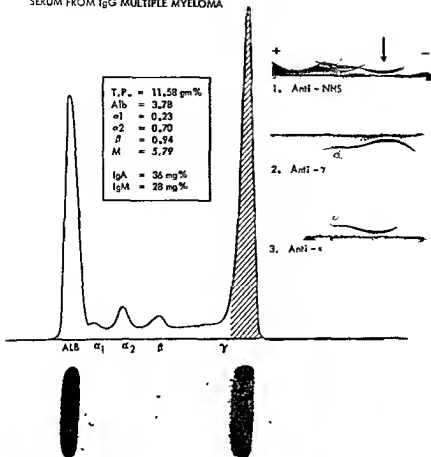
Fig 52 1 Electrophoretic (cellulose polyacetate, Gelman Sepharose III system) and immunoelectrophoretic patterns of sera from various patients. A normal, B polyclonal hypergamma globulinemia

Abbreviations for antisera used in developing immunoelectrophoretic patterns: anti-NHS = anti-normal human serum; anti- $\gamma$  = anti- $\gamma$  heavy chain; anti- $\alpha$  = anti- $\alpha$  heavy chain; anti- $\mu$  = anti- $\mu$  heavy chain; anti- $\kappa$  = anti- $\kappa$  light chain; anti- $\lambda$  = anti- $\lambda$  light chain; anti-L = anti-light chain ( $\lambda$  and  $\kappa$ ). Arrows indicate the location of M-components. See pages 337-339 and Figure 7-22 for further details and techniques.

Special explanatory notes: The IgA myeloma (D) and macroglobulinemia (F) peaks are not as sharp and symmetrical

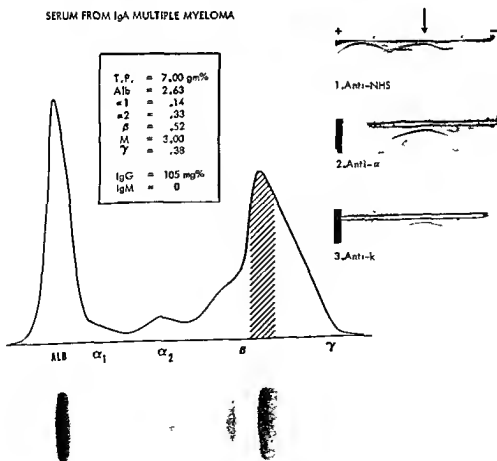
C

SERUM FROM IgG MULTIPLE MYELOMA

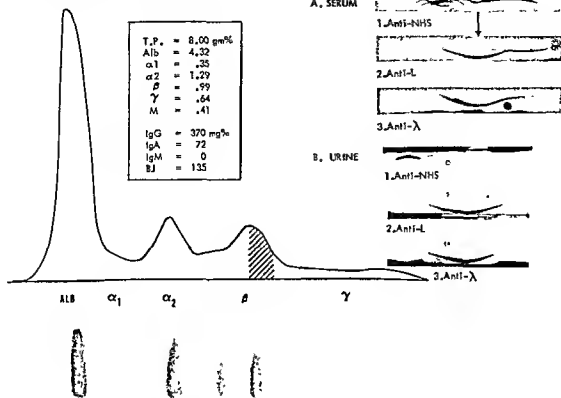


D

SERUM FROM IgA MULTIPLE MYELOMA

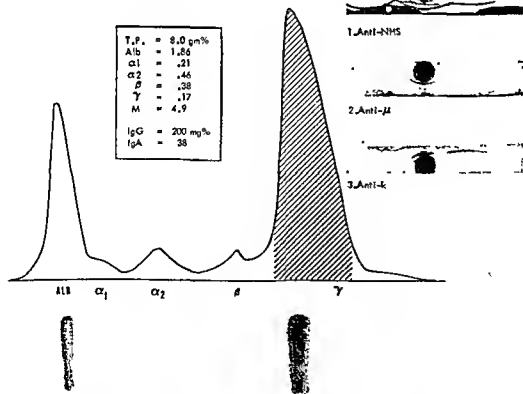


SERUM FROM L-CHAIN MULTIPLE MYELOMA



F

SERUM FROM MACROGLOBULINEMIA



The factors responsible for excessive production of intact M-proteins, the unbalanced production of constituent chains, and the synthesis of altered proteins are unknown. Under normal conditions, synthesis of heavy chains and that of light chains are approximately equal (Chapter 7), and if heavy and light chain synthesis remains balanced in the markedly expanded cell pool of a plasma cell dyscrasia, an intact homogeneous M-component will result. In certain other situations, light chains are produced in excess of heavy chains. This may be due to the proliferation of two separate clones of cells or to a defect in regulatory genes within a single cell line. In some instances, complementary heavy chain production is virtually undetectable ("light chain disease") and, in a few instances, synthesis of heavy and light chains ceases altogether, especially late in the disease (see also page 1613).

Myeloma proteins were at one time considered to be monoclonal "nonsense" proteins without functional significance. However, an ever-growing number of human<sup>25,78,155,269,270</sup> and murine M-components<sup>66,186,187,189,223</sup> with specific antibody activity are now recognized. Thus it is possible that at least some plasmacytomas originate from precursors engaged in natural immune responses and that the M-components of these plasma cell dyscrasias may represent abnormal concentrations of functional antibodies.

Structurally altered proteins and polypeptide chains have been noted with increasing frequency. The most striking examples are seen in patients with "heavy chain disease" that is characterized by M-components consisting of heavy chains with large internal deletions (page 1630). Smaller deletions of parts of the H and L chains have, however, also been demonstrated in apparently intact myeloma proteins. It seems likely that, in all of these instances, the abnormal protein is the result of a defect or mutation within a structural gene.

Some M-components contribute to the clinical manifestations of plasma cell and lymphocyte dyscrasias because of their spe-

cial physicochemical properties. These include (1) precipitation in the cold (cryoglobulins); (2) a high intrinsic viscosity; (3) the ability to complex with other serum proteins such as clotting factors; and (4) the amyloidogenic properties of some light chain fragments. These special properties of immunoglobulins and their fragments will be discussed in the appropriate clinical context. In addition, M-components may increase the catabolism of normal immunoglobulins, thereby leading to low levels of functional immunoglobulins (page 1606).

## Multiple Myeloma

### Definition

Multiple myeloma is a neoplasm of plasma cells, the clinical manifestations of which are dictated by the characteristic bony lesions produced by the tumor, the effects of marrow replacement by tumor tissue, and the pathologic manifestations occasioned by the overproduction of myeloma proteins and their constituent polypeptide chains.

### Incidence

The reported incidence of multiple myeloma has more than doubled during the past two decades,<sup>124,140,150,246</sup> most likely because of the wider use of protein electrophoresis and bone marrow aspiration in diagnosis.<sup>150</sup> Cases have been reported from all parts of the world and no race is known to be immune. In the United States the incidence in Negroes is at least twice that in Caucasians.<sup>140,150</sup> Reported incidence rates are about one to two per 100,000 population in whites and two to four per 100,000 population in blacks per year.<sup>150</sup> While myeloma may be diagnosed as early as the third decade of life the incidence increases with age and reaches a peak during the seventh decade.<sup>150</sup> The disease is probably somewhat more common in males than in females.<sup>176</sup>

## Clinical Manifestations

It is now well recognized that the clinically apparent stage of multiple myeloma is usually preceded by an asymptomatic period of variable duration<sup>90,123,241,253,265</sup> and a few instances of asymptomatic myeloma lasting for two decades have been reported.<sup>165</sup> During this time an elevated erythrocyte sedimentation rate, an M-component on serum electrophoresis, or unexplained proteinuria may be the only manifestations of the disease. As the illness progresses, recurrent infections, weakness, and weight loss become prominent features, to be followed by symptoms resulting from the presence of skeletal lesions and, ultimately, from the development of chronic renal disease.

Increased susceptibility to infection is a common initial finding and constitutes the major cause of death.<sup>258</sup> Myeloma patients characteristically suffer from repeated bouts of sepsis, usually due to high-grade encapsulated organisms such as pneumococci,<sup>83,230,271</sup> but infections due to gram-negative organisms are becoming increasingly prevalent.<sup>156</sup> Thus the patterns of sepsis in multiple myeloma<sup>71</sup> resemble those seen in children with sex-linked agammaglobulinemia (Chapter 44) and in patients with other hematologic neoplasms who have decreased serum levels of normal immunoglobulins (Chapters 44 and 54).

The increased susceptibility to infection is attributable to several factors, the most important of which are low levels of normal immunoglobulins due to (1) defective antibody synthesis,<sup>49,71,92,113,149,271</sup> and (2) an increased rate of gamma globulin catabolism<sup>135,232</sup> (Chapter 44). As a result, normal immunoglobulin levels are almost always decreased and are often less than 20% of normal.<sup>149</sup> These deficits involve all immunoglobulin classes, irrespective of the type of M-protein produced by the malignant cells. Susceptibility to bacterial sepsis is further increased by (3) the frequent occurrence of severe granulocytopenia that occasionally is due to marrow replacement by tumor cells

but, more commonly, is a result of cytotoxic chemotherapy. In addition, functional defects of granulocytes have been reported.<sup>180</sup> Cellular immunity (Chapter 7) is less severely affected, although a delayed rejection time of skin allografts<sup>131</sup> and defective *in vitro* responses of lymphocytes have been reported by some,<sup>93,215</sup> but not by others.<sup>98</sup> In keeping with these findings, myeloma patients are not particularly susceptible to viral infections, with the possible exception of localized and disseminated herpes zoster.<sup>157,170</sup>

The skeletal lesions come to light because of the discovery of a swelling or because of local tenderness, unrelenting pain, or a pathologic fracture. Myelomatous tumors characteristically are multiple and are mainly confined to the sites of red marrow: the ribs, sternum, spine, clavicles, skull, or the extremities about the shoulder or pelvic girdle. Proptosis is a common presenting sign when there is orbital involvement.<sup>207</sup> Palpable swellings generally range from the size of a pea to that of a hazelnut and are elastic and yielding. Parchment-like crepitations may sometimes be elicited. More commonly, however, the only evidence of tumor detected by physical examination is an area of bony tenderness.

Initially the pain is often "rheumatic," wandering, and intermittent and most commonly involves the back, less often the chest or extremities. Girdle sensation or radiation down the legs may be present. The pain may be very severe and may last for hours, days, or longer, but intermittency and even prolonged remissions are common.<sup>83</sup>

Pathologic fractures are frequent, but since they are usually confined to the trunk, they may not be recognized as such initially, but, instead, their manifestations may be considered to be due to pleurisy or neurologic disease. Thoracic deformity is a common end result of multiple fractures of the ribs and sternum, and height may be decreased by several inches because of recurrent compression fractures of thoracic and lumbar vertebrae.

Characteristic bone changes may be dem-



Fig. 52-2 Punched-out lesions in the skull of a patient with multiple myeloma

onstrated by roentgenograms which show rounded, punched-out areas (Figs. 52-2, 3, 4) in the sites already mentioned. Periosteal reactions and spontaneous new bone formation are rare,<sup>104</sup> but may occur with successful therapy.<sup>208</sup> In the ribs the osteolytic lesions frequently have the appearance of diffuse mottling, while in the spine they are evidenced by rarefaction, globular tumor formation, shortening and twisting of the vertebral column, and disappearance of intervertebral discs. Similar multiple, small, discrete, osteolytic lesions may be produced by metastatic carcinoma of the breast or thyroid gland or by other conditions.

In some patients, especially early in the disease, diffuse osteoporosis may be seen instead of discrete punched-out lesions, and in an increasing number of patients no bony lesions whatsoever are demonstrable at the time of diagnosis.<sup>95</sup> Nevertheless, when microradiographic rather than standard radiographic techniques are used, generalized thinning and destruction of trabeculae may be demonstrated.<sup>237</sup> Similar changes are noted in histologic studies. Most patients

without lytic changes on initial presentation eventually develop typical punched-out lesions.

Very rarely, osteosclerosis is described in association with untreated myeloma,<sup>3,73,167,252</sup> but usually other unusual features such as evidence of extramedullary hematopoiesis<sup>73</sup> and polyneuropathy<sup>3,167</sup> are present as well. Therefore, in some instances at least, the atypical bone changes may be due to some other disease, such as myelofibrosis,<sup>73</sup> rather than multiple myeloma.

A solitary plasma cell tumor of bone is found in perhaps 2 to 10% of the patients.<sup>170</sup> It appears most often as a cystic, soap-bubble-type lesion that is much larger and more irregular than the small, discrete lesions characteristic of "multiple" myeloma. At other times it has the appearance of a solitary, destructive lesion within the medullary portion of the bone.<sup>39</sup> Very rarely, new bone formation is seen. Although solitary lesions have in the past been regarded as comparatively benign, most patients have M-components in their serum, and, when followed for long periods of time, most develop diffuse



Fig 523 Lesions about the shoulder girdle in a patient with multiple myeloma (Courtesy of Dr David Bragg)

plasma cell myeloma,<sup>42</sup> even when the original lesion was radically excised or irradiated. Before a diagnosis of solitary plasmacytoma is made, areas of bone marrow at a distance from the single lesion should always be ex-

amined and careful, long-term clinical follow-up is essential.

"Rheumatoid" manifestations are sometimes observed.<sup>56</sup> These have been attributed to the deposition of amyloid in and about the joints. Occasionally, however, true rheumatoid arthritis and related joint diseases have preceded or have occurred simultaneously with multiple myeloma.<sup>86,256,264</sup> Their possible relationship to the development of multiple myeloma has been mentioned already (page 1600).

Involvement of the *nervous system* by multiple myeloma is largely a function of its proximity to skeletal structures. Thus root symptoms are common, and paraplegia due to compression of the spinal cord may occur. The latter is an extremely serious complication and usually requires immediate surgical decompression and local radiotherapy. Occasionally, cranial nerves may also become involved by tumor tissue. Root symptoms and peripheral neuropathies due to infiltration of these structures by amyloid have been described,<sup>249</sup> as have occasional instances of polyneuropathy and proximal myopathy in the absence of direct invasion of these structures by amyloid.<sup>41,170,230,249,254</sup> Such polyneuropathy appears to be similar to that seen in association with other neoplastic diseases. Multifocal leukoencephalopathy also has been reported.<sup>58</sup>

One of the most striking neurologic manifestations encountered in patients with multiple myeloma is *hypercalcemic encephalopathy*. This may present as confusion, delirium, or coma, usually following a period of weakness, lethargy, nausea, vomiting, and dehydration.<sup>69</sup> Such neuropsychiatric symptoms are more common in myeloma than in other diseases characterized by hypercalcemia, such as hyperparathyroidism.<sup>60,116</sup> About half of the patients with hypercalcemia develop EEG changes consisting predominantly of diffuse slowing and delta episodes, suggesting a disturbance of brain stem function.<sup>69</sup> While the relationship between hypercalcemia and central nervous system dysfunction is not a precise one<sup>68,100</sup> and some patients with high



Fig 52-4 Pelvic lesions in a patient with multiple myeloma

serum calcium have no obvious mental symptoms, whereas others with relatively lower calcium levels do, therapeutic measures designed to alleviate the hypercalcemia usually have a favorable effect on the neurologic manifestations. It is unclear to what extent other factors such as disturbed kidney function and acidosis contribute to the neuropsychiatric manifestations (see also Chapter 54).

*Extraosseous tissues* are frequently involved in multiple myeloma.<sup>39,179</sup> When both macroscopic deposits and microscopic infiltrates are included, extraosseous involvement may be seen in about two thirds of autopsied subjects.<sup>179</sup> Patients with the longest duration of illness tend to have the most widespread extramedullary disease. The commonest sites of involvement are the spleen,<sup>179,226</sup> liver,<sup>64,179,230</sup> lymph nodes,<sup>50,179,240</sup> and kidneys,<sup>179</sup> but infiltrates may also be encountered in most other tissues, such as thyroid and adrenal glands, ovary, testis, lung, pericardium, and intestinal tract.<sup>88,227</sup> Palpable hepatomegaly was reported in 40% of patients in

one series<sup>230</sup> and in 26% in another,<sup>2</sup> whereas hepatosplenomegaly<sup>230</sup> or splenomegaly<sup>2</sup> was seen only in about half that number. Discrete "solitary" plasma cell tumors may develop in soft tissues as well as in bone, most commonly in the respiratory tract or the oral cavity,<sup>179</sup> but they have also been described in the kidney, ovary, and intestine, or even the spleen.<sup>33</sup>

*Chronic renal failure* frequently becomes a prominent feature of the disease and is the end result of a number of interrelated factors.<sup>224</sup> Of prime importance is the filtration of large amounts of Bence Jones proteins by the glomeruli,<sup>39,47</sup> thereby presenting the proximal tubules with a massive reabsorptive load. In time, the tubules accumulate proteinaceous inclusion bodies, and cellular degeneration and impairment of tubular function soon follow.<sup>132,138</sup> The hyaline inclusion bodies within the epithelial cells have been shown to have the immunofluorescent staining properties of light chains.<sup>132</sup> In addition, large obstructing casts form along the entire length of the tubule, leading to disten-



sion and ultimate destruction of the whole nephron. These obstructing casts have been shown to contain albumin, fibrinogen, and  $\gamma$ ,  $\kappa$ , and  $\lambda$  chains, but no  $\beta_2$  globulin, IgM, or IgA.<sup>132</sup> Others have demonstrated the presence of amyloid-like fibrils within these casts and have suggested that they arise through a process of light chain degradation.<sup>83a</sup> The combination of hyaline or cancellated tubular casts usually surrounded by a syncytium of epithelial cells, accumulated hyaline droplets in epithelial cells, tubular atrophy, and, frequently, interstitial fibrosis and nephrocalcinosis constitutes the typical picture of 'myeloma kidney'.<sup>122a</sup>

Specific tubular reabsorption defects, including the adult Fanconi syndrome, have also been described as part of the nephrotoxic spectrum of Bence Jones proteins.<sup>57,67,94,110</sup> This suggests that some of these proteins have the capacity to interfere with specific tubular transport mechanisms. It is not known why some Bence Jones proteins are nephrotoxic while others are not.

In addition to Bence Jones proteins, several other factors are important in causing renal dysfunction.<sup>13 170 224</sup> Thus, *hypercalcemia* and *hypercalcaemia* due to bony destruction and immobilization may lead to all the manifestations of hypercalcemic nephropathy, while *hyperuricemia* (Chapter 54) due to increased cellular turnover, frequently aggravated by therapy, results in deposition of uric acid crystals in the distal tubules, collecting ducts, and ureters.

Sometimes acute renal failure occurs because of aggravation of chronic renal disease by dehydration or other insults. Thus intravenous pyelography is known to be associated with acute renal shutdown,<sup>129,170</sup> presumably because of the dehydration induced in preparation for this procedure. Of equal pathogenetic importance may be the capacity of some contrast media to induce Bence Jones protein precipitation in acid urine.<sup>129</sup>

Occasionally, *renal amyloidosis* complicates multiple myeloma. This will be discussed in a subsequent section (page 1636).

## Effects of Abnormal Proteins

While most of the clinical manifestations of multiple myeloma reflect the presence of malignant disease rather than the presence of abnormal proteins, some patients suffer from symptoms caused by the very large amounts of abnormal proteins, or proteins with unusual physicochemical properties.

*Undue sensitivity to cold* may be produced by myeloma proteins or protein complexes that undergo reversible precipitation at low temperatures. Such proteins are referred to as *cryoglobulins* (page 1640) and are most often associated with multiple myeloma and other plasma cell and lymphocyte dyscrasias. While patients with such proteins frequently are asymptomatic<sup>153</sup> they may have symptoms of cold urticaria, acrocyanosis, tingling, numbness, and a true Raynaud's phenomenon. Occasionally, trophic changes of the extremities, gangrene of digits, hemorrhagic manifestations, and thromboses of major blood vessels occur.<sup>170</sup>

The *hyperviscosity syndrome* (page 1625) is due to the presence of serum proteins with a high intrinsic viscosity. Most commonly this is seen in primary macroglobulinemia (Chapter 53), but occasionally it occurs in multiple myeloma with IgG or IgA proteins.<sup>21,119,139,196,234,239,259,262</sup> The high viscosity interferes with efficient circulation to the brain, eyes, kidneys, or digits. As a result, the fundi may show a very characteristic appearance with dilated venules and many hemorrhages; confusion and more severe central nervous system disturbances may develop quite suddenly. Signs of progressive peripheral vascular and cardiac insufficiency may also result from impaired circulation in the small capillaries. The diagnosis and treatment of this syndrome are discussed in Chapter 53.

*Hemorrhagic manifestations*, when not due to thrombocytopenia, are most commonly due to the ability of some M-components to interact with various proteins, including coagulation factors.<sup>44,127,159,163,180 239,251</sup> This type of complexing has been shown to in-

volve factors V, VII, and VIII, and prothrombin and fibrinogen. Abnormal fibrin aggregation and ultrastructure may be seen.<sup>44,127,194</sup> Increased factor VIII activity also has been reported.<sup>257</sup> In some patients, M-components may lead to abnormal platelet aggregation and function.<sup>44,159,181</sup> The nature of these defects is discussed in Chapter 35.

The immunochemistry and clinical manifestations of amyloidosis are discussed in Chapter 53 (page 1633). It is likely that amyloid associated with plasma cell dyscrasias is made up of fragments of light chains. Only about 10% of patients with multiple myeloma develop amyloidosis and the spectrum of disease ranges from cases with minimal, clinically insignificant deposits to those in which amyloidosis is the dominant feature. Amyloidosis appears to be particularly common in patients with IgD myeloma.<sup>72,80,103,266</sup>

### Associated Diseases

An unusually large number of patients with multiple myeloma appear to develop a second malignant disease, most commonly one involving the breast, biliary system, or bowel.<sup>170,244,258</sup> An apparently increased incidence of hematologic malignant conditions also has been reported, including acute myeloblastic, myelomonocytic or monocytic leukemia,<sup>125,172,193,222,248</sup> chronic myelocytic leukemia,<sup>204</sup> Kaposi's sarcoma,<sup>146</sup> and thymoma.<sup>133</sup>

### Laboratory Manifestations

#### Blood

Most patients with multiple myeloma eventually develop anemia that is usually of moderate severity, but may be severe; hemoglobin levels between 7 and 10 g/dl are most commonly found. The red cells in most of the patients are normochromic and normocytic and rouleaux formation may be prominent. The reticulocyte count is low. In most instances, defective red cell production can be demonstrated (Chapter 54). Occasionally, increased plasma volumes result in fictitiously

low hematocrits.<sup>120</sup>

While a normochromic, normocytic blood picture is the rule, exceptions have been noted. Thus the red cells are sometimes macrocytic and there may be megaloblastic changes in the bone marrow.<sup>32,105,128</sup> This may be due to folate deficiency,<sup>105</sup> or, occasionally, to true vitamin B<sub>12</sub> deficiency.<sup>32,128</sup> In other cases the macrocytosis has no discernible cause and such patients do not respond to specific vitamin therapy. Occasionally, when there is intestinal bleeding due to defective coagulation, amyloid infiltrates, vascular damage, or some other cause, iron deficiency anemia may develop. Polycythemia has also been reported in association with multiple myeloma,<sup>36,79,130,236</sup> but the exact relationship between these disease entities is unknown.

Enumeration of red cells may be difficult because of clumping and it may be impossible to make satisfactory blood smears because of rouleaux formation. This is usually due to the increased amount of globulin in the plasma and, in such an instance, dilution with normal saline or Gower's solution may provide a more satisfactory preparation. Because of clumping and rouleaux formation, blood grouping may be difficult. A rapid erythrocyte sedimentation rate is characteristically associated with red cell clumping and rouleaux formation, but, in the presence of cryoglobulins, the sedimentation rate may be zero.

The total leukocyte count usually is normal before the start of chemotherapy, but in some patients leukopenia may be present. Rarely the count is greatly increased.<sup>210</sup> The differential count may reveal no abnormality but in up to 50% of patients a mild neutropenia and a relative lymphocytosis are present and immature-appearing lymphocytes and plasma cells may be seen. The latter are common if a careful search of concentrated preparations is made.<sup>84</sup> In a few cases, numerous plasma cells have been observed, especially in the later stages of the disease. In one of our patients, 84% of the  $29.0 \times 10^9/l$  leukocytes were myeloma cells. The condition in such

a patient has been referred to as *plasma cell leukemia*.<sup>20,77s,114,168,197</sup> Its clinical course ranges from that of the terminal leukemic stage of otherwise classic multiple myeloma to an a priori fulminant course of acute leukemia,<sup>197</sup> characterized by hepatosplenomegaly, weakness, severe anemia, and bleeding manifestations. The protein abnormalities are similar to those of classic myeloma but the clinical course is generally acute or subacute and, except in rare cases,<sup>11</sup> no response to chemotherapy is observed.<sup>197</sup>

The platelet count usually is normal but sometimes is low. When a prolonged bleeding time is demonstrated in conjunction with moderate thrombocytopenia, it is more often due to a drug or M-component-induced functional platelet defect, or a vascular defect associated with cryoglobulins or the hyper-viscosity syndrome than to thrombocytopenia (Chapter 53)

### Bone Marrow

The characteristic findings are the "myeloma cells" (Plates VII, XXII, XXIII) which are present in virtually all patients with myeloma.<sup>14, 61, 170, 230, 232</sup> Occasionally a careful search is necessary to find these cells; this is probably attributable to the uneven way in which myeloma involves the marrow, especially in the early stages of the disease. Usually the marrow contains at least 5 to 10% myelomatous plasma cells, and when the number exceeds 15 or 20% the diagnosis is highly probable, especially if the cells occur in sheets similar to those characteristic of other types of tumor cells in the marrow. Nevertheless, the marrow picture must never be interpreted in the absence of other evidence of myeloma, since occasionally a similar picture may be seen in reactive plasmacytosis of various causes.

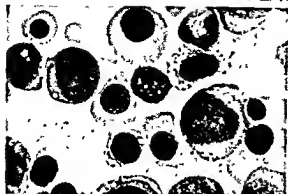
Typically the *myeloma cell* is moderately large (15 to 30  $\mu$ m), round, or ovoid, and contains a nucleus about 5 to 7  $\mu$ m in diameter. Occasionally myeloma cells are extraordinarily large and contain two or three nuclei. The nucleus is round, eccentrically placed,

and contains one or sometimes two nucleoli. The chromatin is not as fine as in the myeloblast nor as coarse as in the normal plasma cell, nor is the wheelspoke arrangement of the latter present. The cytoplasm may be basophilic and bright blue, or lighter in color.

There is, however, a great deal of variability from case to case (Plate XXII, E, F, G; Plate XXIII, F) as well as in the same case, and the cells may range from very anaplastic, immature forms to cells closely resembling normal plasma cells. At least part of the morphologic variability depends on the degree of functional differentiation of the cells. As the cells mature and develop the ability to synthesize gamma globulin, there is progressive accumulation of cytoplasmic RNA which is responsible for the characteristic basophilia and pyroninophilia of the cytoplasm. Such cells have a highly developed endoplasmic reticulum<sup>31,59</sup> and gamma globulins can be demonstrated within the endoplasmic cisternae.<sup>59,203</sup> Average molecular synthesis rates of 12,500 to 85,000 IgG molecules per minute per myeloma cell have been calculated.<sup>216</sup>

As in normal plasma cells, but particularly in those of inflammatory exudates, various types of inclusions may be seen in myeloma cells<sup>31,143</sup> (Plates VII, XXII). These include hyaline cytoplasmic spherules (*Russell bodies*), intranuclear inclusion bodies (similar hyaline spherules), and eosinophilic and PAS positive *granules*. All of these structures share a common electron density and osmophilic appearance when viewed under the electron microscope, and in most instances represent accumulations of gamma globulin. They are found in the nucleus, the Golgi apparatus, the intercisternal spaces, and inside the cisternae. Because of the light and electron microscopic identity of Russell (intracytoplasmic) and intranuclear inclusion bodies, it has been suggested that the latter are probably also of cytoplasmic origin.<sup>143</sup> "Mott cells," "grape cells," (Plate VII, H), and "morular cells" appear to be aggregations of Russell bodies. Some plasma cells also seem capable of phagocytosis.<sup>1</sup>

# PLATE XXII



A



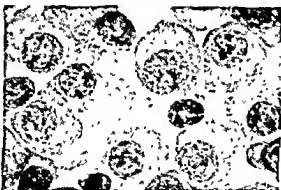
B



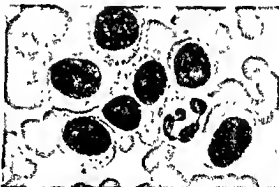
C



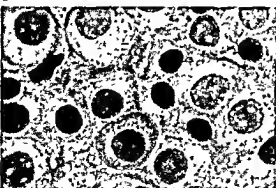
D



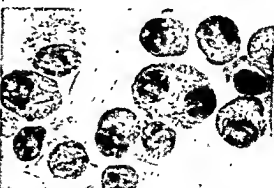
E



F



G



H

*Erythroleukemia and multiple myeloma (bone marrow and blood,  $\times 1000$ )*

A, B, C, D, Erythroleukemia, bone marrow A, B, Wright's stain C, D, PAS stains In B a giant multinucleated erythroblast is shown

E, F, G, H, Multiple myeloma E, IgG myeloma, "mature" cell type, F, "immature cell" type, G, cells with "flaming" type cytoplasm, H, crystalline material in cytoplasm of myeloma cells

tempts have been made to correlate the hologic characteristics of myeloma cells the type of abnormal protein seen.<sup>61,142,178,233</sup> Such attempts are based on chemical differences between IgG and proteins and, particularly, on the higher hydrate content of the latter. In one of 72 patients with various plasma cell neoplasias, "flame cells" (Plate VII, J) and "giant cells" ("thesauocytes") (Plate VII, M) found only in those with IgA myeloma.<sup>178</sup> Others have partially confirmed findings.<sup>32,142,144</sup> The storage material consists of IgA globulin, possibly highly polymerized and therefore incapable of being degraded normally.<sup>245</sup> In addition to myeloma cells, histiocytes, monocytes, and lymphoid cells with plasmoid features have been described in the marrow of patients with multiple myeloma,<sup>141</sup> and occasionally this may make differentiation from some cases of lymphoma (see 51) difficult. Chromosome abnormalities<sup>113,34</sup> were discussed in Chapter 46.

### Immunologic Abnormalities

Typical M-components and/or Bence Jones proteins are demonstrable in virtually all patients with multiple myeloma. When they are not found, the adequacy of the study may be questioned.<sup>18,23,101,173,268</sup> Occasional myelomas without M-components have been reported from good laboratories<sup>48,60,111,173,205</sup> and the incidence appears to be less than 1%. Methods for the detection of M-components described earlier (page 1601). The cells are "non-secreting" have the same appearance as those of patients with other forms of myeloma, even as judged by electron microscopy. The question has been raised as to whether the non-secreting may be secreting protein not recognized by currently used methods.<sup>16</sup> The distribution of various immunoglobulin classes among myeloma proteins is usually proportional to the concentration of

their normal counterparts in the serum (Table 7-3). In most series,<sup>18,101,173,244</sup> the breakdown is approximately as follows: 50% IgG, 25% IgA; 25% have light chains only. IgD myelomas account for about 1% of all cases, and IgE<sup>77,114,168</sup> and IgM<sup>101</sup> myelomas are exceedingly rare. Thus only 75% of myeloma patients show prominent serum "spikes," light chains being much more difficult to demonstrate in the serum. The simultaneous presence of two M-components, most commonly IgG and IgA, has been documented in a small number of cases.<sup>17,52,209,211,221</sup>

Among IgG, IgA, and Bence Jones proteins,  $\kappa$  and  $\lambda$  chains are about equally distributed, but IgD myeloma is almost always associated with  $\lambda$  chains.<sup>72,76,102,103,193,266</sup>

Some interesting clinical correlations have been established for the various immunologic classes of myeloma.<sup>101</sup> Thus IgG myelomas appear to be associated with a greater reduction of normal immunoglobulins, more frequent infections, a higher serum level of M-protein, apparently slower tumor growth rates, and less hypercalcemia and amyloidosis than do other types. One of the outstanding features of IgA myeloma is hypercalcemia, and complicating infections are somewhat less frequent than in other varieties. Amyloidosis is not uncommon. IgD myelomas often occur in younger patients, Bence Jones proteinuria is usually marked, and hypercalcemia, amyloidosis, and renal failure are prominent features. Bence Jones myelomas are said to grow fastest of all and are associated with more osteolytic lesions, more hypercalcemia, and a higher incidence of renal failure and amyloidosis than either the IgG or IgA varieties. Survival of such patients is said to be relatively poor,<sup>37</sup> but this may simply reflect later detection of the disease. It has been suggested that myeloma without detectable M-components is even more poorly differentiated than Bence Jones myeloma and carries the worst prognosis of all.<sup>101</sup> Severe reduction of normal immunoglobulins is the rule.

Special protein properties such as cryoprecipitation, high intrinsic viscosities, and

amyloid deposition have been discussed elsewhere (page 1610).

### Other Laboratory Features

Many patients give evidence of renal failure with *azotemia*, high serum creatinine, and low creatinine clearance rates. The urine may contain, in addition to Bence Jones proteins, albumin, casts, and renal epithelial cells, but it is unusual to find red cells.<sup>141</sup> The concentrating functions of the kidney also may be impaired. In patients suffering from the Fanconi syndrome (page 1610), the urine may contain large amounts of amino acids, sugar, and phosphates. *Hyperuricemia* is a frequent complicating feature, as is *hypercalcemia*. The alkaline phosphatase is either normal or only slightly elevated.

### Diagnosis

The cytologic, clinical, and laboratory criteria for the diagnosis of multiple myeloma are listed in Table 52-1. In order to qualify for the diagnosis of multiple myeloma a patient must have one of the following combinations of criteria:<sup>51</sup>

1a and 1b

1a or 1b and either 2a, 2b, 2c or 2d.

Table 52-1 Criteria for the Diagnosis of Multiple Myeloma<sup>51</sup>

1	Cytologic criteria
a	Marrow morphology. Plasma cells and/or myeloma cells in excess of 10% when 1000 cells or more cells have been counted
b	Biopsy proven plasmacytoma either in bone or soft tissues
2	Clinical and laboratory criteria
a	Myeloma protein (M-component) demonstrable by electrophoresis of plasma
b	Myeloma protein (M-component) demonstrable by electrophoresis of urine
c	Roentgenologic evidence of osteolytic lesions. Generalized osteoporosis qualifies as a criterion if the marrow contains in excess of 30% plasma or myeloma cells
d	Myeloma cells in at least two peripheral blood smears

In addition, other diseases commonly giving rise to plasmacytosis ("collagen vascular" and immune complex diseases, cirrhosis, metastatic carcinoma, viral exanthems, should be excluded. Furthermore, if disease of bone is not evident, other features associated with myeloma, such as otherwise unexplained anemia, should be present. The presence of amyloid disease does not mitigate against the diagnosis of multiple myeloma.

### Treatment

#### General Measures

Patients with multiple myeloma need more support from their physicians than do most other cancer patients—severe pain is a common and almost continuous problem and there may be pathologic fractures; bacterial infections recur with distressing frequency and anemia, renal disease, and other manifestations may be troublesome; in addition, the disease is almost invariably fatal. In order to ease the patient's lot it is important to call attention to the improving prognosis with therapy, and it is necessary to alleviate exaggerated fears of pathologic fractures and to stress the dangers of immobilization with the consequent further skeletal demineralization and hypercalcemia. Above all, the physician must show a sincere and continuing interest in the patient and his inevitably recurring complaints and problems.

*Mobilization* is of prime importance, but is often made difficult by pain, fractures, and fear. The milder forms of pain frequently respond to salicylates and/or codeine, but sometimes more potent analgesics such as meperidine or opiates are necessary. However, salicylates must not be used when the platelet count is low or when platelet function is impaired. When pain is due to a readily identifiable bone lesion, *local* radiotherapy will almost always alleviate it (see below). *Orthopedic supports* for the spine, the rib cage, or the extremities often are helpful, but must be used for as short a time and as sparingly as possible, since they always lead to

some immobilization, disuse atrophy, and, eventually, habituation.

*Sodium fluoride* therapy has been used in the treatment of myeloma, since its chronic administration leads to positive calcium balance, increased mineralization of bone, and decreased pain in patients with various metabolic disorders of bone.<sup>30,199,202</sup> However, contrary to the initial reports,<sup>45,46</sup> double blind studies have demonstrated no beneficial effects of a subjective or objective nature.<sup>91</sup> Fluoride therapy undoubtedly leads to increased skeletal mineralization but this is due to the production of many layers of an inferior, fluffy bone that appears more sclerotic than normal,<sup>91</sup> but is, in fact, weaker.<sup>154</sup> Indeed, fluorosis may be detrimental to the clinical course of patients with multiple myeloma.<sup>91</sup> In addition, fluorides have toxic side effects which include nausea, diarrhea, gastrointestinal bleeding, skin rashes, and arthralgias. For all of these reasons the use of fluorides is not recommended.

### *Irradiation Therapy*

There is no evidence that radiotherapy influences the duration of survival but, as pointed out earlier, it remains the treatment of choice for individual painful bone lesions that do not respond to chemotherapy, for pathologic fractures, and for lesions that impair the function of vital structures. Relief of pain is prompt, pathologic fractures may heal, and impending compression of the spinal cord can be prevented.<sup>19,39,106</sup> Unfortunately, patients frequently return in a short time with trouble in another area and, while radiation may be given again, its use is ultimately limited by the development of bone marrow suppression, which also interferes with effective chemotherapy. Thus it is important to restrict the field of therapy and the total administered dose as much as possible, especially when the aim is relief of pain, rather than the treatment or prevention of a pathologic fracture. Total doses of less than 500 rads are frequently effective and seldom is it necessary to exceed 1500 rads.

### *Chemotherapy*

Since multiple myeloma is a generalized disease, systemic chemotherapy is the treatment of choice. Two alkylating agents, melphalan and cyclophosphamide, presently hold the greatest promise and are probably equal in their effectiveness.<sup>74,206</sup> The pharmacology and mode of action of these drugs are discussed in Chapter 55.

Most studies show that the proper use of these agents results in subjective and objective improvement in a majority of patients.<sup>51,74</sup> *Subjective changes* include significant pain relief, often within a few days of the start of therapy, and concomitant improvement in the patient's overall performance status as measured, for instance, on the Karnofsky scale.<sup>115</sup> *Objective changes* consist of (1) a significant decrease (>50%) in the concentration of M-components, including Bence Jones proteins<sup>51,74</sup>; (2) a decrease in the myeloma cell mass (see next paragraph);<sup>214</sup> which may be reflected in a lower percentage of marrow myeloma cells,<sup>51,74</sup> although the irregular distribution of the cells in the bone marrow sometimes makes interpretation of marrow data difficult; (3) reduction in the size of soft tissue masses<sup>148</sup>; and (4) repair of osteolytic lesions, x-ray-demonstrable recalcification of bone, or a general increase in bone density.<sup>7,71,108,208,252</sup> It must be stressed, however, that the radiographic appearance of bones may worsen initially and healing may not be evident for at least 6 to 12 months.<sup>74</sup> This initial radiographic deterioration must not be interpreted as lack of response to therapy, especially if other parameters indicate improvement. (5) Other objective parameters of improvement include a rise in hemoglobin levels or a decrease in transfusion requirements<sup>7,51,74</sup> and at least partial correction of hypercalcemia and uremia<sup>148</sup> as well as the normalization of immunoglobulin levels.<sup>3</sup> (6) Although groups of untreated patients have not been compared in a controlled study with patients receiving currently available forms of therapy, it does appear that the survival time of patients re-

sponding to melphalan or cyclophosphamide has been significantly lengthened.<sup>8,51,74,108,148</sup> The number of complete remissions unfortunately remains distressingly low; thus better modes of therapy will have to be developed.

There is a close relationship between the total tumor cell mass and the total body synthetic rate of M-components.<sup>242</sup> Changes in the latter can therefore be used to estimate the effectiveness of chemotherapy in slowing or arresting the rate of tumor growth. For practical purposes the measurement of changes in the concentration of serum M-components is a useful substitute<sup>74,105,118,242</sup> although such measurements frequently underestimate the changes in tumor size.<sup>242</sup> Since Bence Jones proteins have a much shorter half-life than do intact myeloma proteins (<1 day, versus 14 to 21 days for IgG, for example), a significant decrease in Bence Jones proteinemia and proteinuria is usually seen long before similar changes are evident in the concentration of intact myeloma proteins. A concomitant increase in the level of normal immunoglobulins is seen in only 20 to 30% of responding individuals,<sup>5</sup> and no increase occurs in patients refractory to therapy. It is of special interest that patients with a slow response, as measured by the drop in M-component concentration, seem to have a much better prognosis than those in whom a rapid drop in myeloma proteins is seen.<sup>100</sup> The latter group was found to fare little better than patients who did not respond at all.<sup>100</sup> With the exception of one report<sup>27</sup> there appears to be no consistent correlation between the L-chain type and response to therapy.<sup>8,171,206</sup>

*Melphalan* (L-phenylalanine mustard) has been used most extensively,<sup>7,26,38,51,62,74,108,109</sup> either alone or in combination with other agents, such as prednisone. Objective and subjective improvement is seen in about one third to two thirds of treated patients, depending in part on patient selection, the expertise of the physician, and, perhaps, on the mode of administration.<sup>8</sup>

Melphalan may be given either continuously or intermittently. Although the best

response rates reported for either method are similar,<sup>8,74,108,109,148</sup> comparative studies made within a single institution attest to the superiority of intermittent schedules, especially when melphalan has been combined with a second agent such as prednisone.<sup>8,82</sup>

When melphalan is used *intermittently*, a dose of 0.25 mg/kg/day is given for four days in conjunction with prednisone (2.0 mg/kg/day, also given for four days).<sup>8,82</sup> The treatment schedule is repeated every six weeks. The addition of procarbazine to this treatment program does not appear to increase the response rate significantly, or the duration of remission or survival.<sup>9</sup>

In *continuous regimens* the patient is generally started on 8 to 10 mg melphalan per day for seven to ten days.<sup>74</sup> The dose is then reduced to about 2 mg daily, but upward and downward adjustments in dose must be made, depending on the sensitivity of the normal marrow elements to the drug. A rest period of at least two weeks following the initial course of high-dose therapy has been recommended by some,<sup>108</sup> but others have warned of the danger of tumor and M-component rebound during this period.<sup>74</sup> Some clinics prefer to start with an initial dose as low as 4 mg daily and have reported results similar to those obtained with larger amounts of drug.<sup>148</sup>

The most important complication of melphalan therapy is bone marrow toxicity (Chapter 55). Other toxic effects are unusual, although anorexia, nausea, vomiting, and alopecia have been mentioned.<sup>206</sup>

*Cyclophosphamide*, another alkylating agent, is probably as effective as melphalan in the therapy of myeloma.<sup>11,38,121,122,151,206,220,228</sup> Dosage schedules vary considerably, ranging from 1.5 mg to 4.0 mg/kg/day. Intermittent cyclophosphamide therapy has been tried<sup>23,29</sup> and while it is clearly effective in the treatment of myeloma, the comparative value of continuous and intermittent therapy has not been tested. When used *intermittently*, 1 g/m<sup>2</sup> is given intravenously as a single "push" or 0.25 g/m<sup>2</sup>/day may be given by mouth on four consecutive



days. The treatment is repeated every three weeks. When giving cyclophosphamide in large doses, an adequate fluid intake (3 to 4 liters daily) must be ensured during therapy in order to decrease the risk of hemorrhagic cystitis and bladder fibrosis (Chapter 55).

At present it is impossible to decide whether melphalan or cyclophosphamide is the drug of choice in multiple myeloma. Bone marrow suppression appears to be approximately equal for both drugs, although the incidence of thrombocytopenia and bleeding may be slightly lower with cyclophosphamide.<sup>206</sup> Alopecia is probably more common with cyclophosphamide than with melphalan, as is hemorrhagic cystitis and eventual bladder fibrosis, but the latter complication can be prevented (see Chapter 55).

While both melphalan and cyclophosphamide are alkylating agents, their respective mechanisms of action probably differ to some degree, since patients resistant to one may still respond to the other.<sup>25,29,74</sup> Prolonged remissions have been obtained with the second agent after the patient had developed resistance to the first.<sup>74</sup>

### Other Agents

When used alone, the daily administration of prednisone is ineffective in the treatment of multiple myeloma.<sup>145</sup> *Intermittent high-dose prednisone therapy* may, however, be useful in patients who are poor treatment risks for alkylating agents because of severe bone marrow suppression or who are no longer responding to other agents.<sup>218</sup> The use of prednisone in conjunction with alkylating agents has already been mentioned.<sup>8,51,82</sup> Whenever possible, steroids should be used in large doses intermittently since continuous or even alternate-day regimens are associated with complications such as cushingoid changes, uncontrolled diabetes, recurrent infection, and gastrointestinal problems.<sup>8</sup> *Urethane* (ethyl carbamate) was used extensively prior to the introduction of alkylating agents and was reported to produce objective changes in 20% of treated pa-

tients.<sup>117,136,170,212</sup> One controlled trial failed to show any benefit,<sup>107</sup> but the results of this study have been criticized by some.<sup>74</sup> Use of the drug has generally been abandoned because of toxic and unpleasant side effects,<sup>170</sup> as well as the availability of better agents.

*Nitrosourea compounds* such as BCNU and CCNU are presently being tested in the treatment of myeloma.<sup>74</sup> *Purine and pyrimidine antagonists* as well as *folic acid antagonists* are ineffective.<sup>28</sup>

### Therapy of Complications

*Hypercalcemia* with its attendant dehydration and, frequently, renal failure requires prompt attention. Dehydration is due to many factors including the obligatory fluid loss of hypercalciuria, the tubular defect due to hypercalcemia, and the frequent nausea and vomiting that accompany this syndrome. The management of hypercalcemia must be prompt and vigorous. (1) The dehydration must be corrected immediately with sufficient parenteral fluids to assure a urine output of at least 2,000 ml/day. This in itself will increase calcium clearance by the kidneys. (2) Increased sodium clearance in man is accompanied by increased calcium clearance<sup>68</sup> and saline infusions may therefore be given, unless the patient is also suffering from congestive heart failure. Sometimes thiazides are used in conjunction with saline infusions. (3) Most myeloma patients with hypercalcemia promptly respond to prednisone in doses of 40 to 60 mg/m<sup>2</sup>/day.<sup>22,132,154,252</sup> If the patient is incapable of taking pills by mouth, equivalent doses of hydrocortisone may be given intravenously. A prompt drop in calcium levels is usually seen; patients suffering from hypercalcemic encephalopathy may show marked and rapid improvement. (4) When patients do not respond to other measures, the administration of inorganic phosphate salts has been recommended.<sup>87</sup> The hypocalcemic effect of phosphates is prompt, often occurring within hours of the intravenous administration of 1 liter of a 0.1 M solution of disodium phosphate and mono-

potassium phosphate. Daily oral therapy with 1 to 3 g of phosphate given as the disodium or dipotassium salt is also effective in maintaining normocalcemia. During phosphate therapy it is important to measure calcium levels frequently in order to prevent hypocalcemia. It may also be necessary to monitor the electrocardiogram for prolongation of the QT interval. The major disadvantages of phosphate therapy are metastatic calcifications,<sup>22a,235</sup> and diarrhea if the salts are taken by mouth. (5) Refractory patients may also be treated with large doses of furosemide intravenously,<sup>241</sup> careful attention being paid to the replacement of fluids and electrolytes. This form of therapy is probably safe and effective when used under close supervision in a hospital, but a good deal of clinical experience is needed.

*Hyperuricemia* and *hyperuricosuria* frequently contribute to renal failure and should be treated with adequate fluids and allopurinol, a xanthine oxidase inhibitor that reduces serum and urine uric acid levels regardless of the underlying cause<sup>178</sup> (Chapter 54). The usual dose is 300 to 600 mg daily. Since effective treatment of multiple myeloma will invariably exaggerate the problems of hyperuricemia, chemotherapy should be withheld until adequate renal function has been restored.

*Immunoglobulin therapy* has been advocated by some as prophylaxis against bacterial sepsis<sup>55</sup> but has been shown to be ineffective,<sup>217</sup> probably because of the increased fractional catabolic rate of normal immunoglobulins in patients with myeloma.

Symptoms due to the hyperviscosity syndrome (page 1625) respond well to plasmapheresis (Chapter 53).

*Spinal cord compression* is an extremely serious complication and requires *immediate* surgical decompression, followed by local radiotherapy in most instances.

## Prognosis

It has always been recognized that the course of multiple myeloma varies greatly.

Survival may be very short but sometimes it is very prolonged, spanning a decade or more with repeated exacerbations and remissions.<sup>38,74,141</sup> One remarkable patient has lived for more than 30 years following recognition of disease in the right ischium, continuing to do well despite other radiologically demonstrable lesions and an IgG M-protein.<sup>177</sup> Since the early 1960's, notable advances have been made in the treatment of multiple myeloma, both in terms of general management as well as through the introduction of effective therapeutic agents. These advances are reflected in the induction of at least partial remissions in as many as two thirds of all patients and perhaps by the prolongation of a comfortable and useful life. Thus, prior to the use of alkylating agents, the median survival time was 17 months from the onset of symptoms and only seven months from the onset of therapy<sup>160</sup>; in contrast, the median survival time is now between 24 and 50 months from the onset of therapy, representing a three- to seven-fold improvement.<sup>74</sup> However, it must be recognized in making this comparison that diagnosis now is made at an earlier stage of disease than it once was.

The blood urea concentration at the time of diagnosis is the single most important factor influencing prognosis.<sup>152</sup> The median survival time of patients with blood urea concentrations of 80 mg/dl or more is only two months, that of patients with concentrations of less than 40 mg/dl, 37 months.<sup>152</sup> Other indicators of a poor prognosis include proteinuria in excess of 40 mg/dl, a serum albumin concentration of less than 3 g/dl, and a hemoglobin of less than 7.5 g/dl. Bence Jones proteinuria correlates with azotemia but may not itself have adverse significance as regards prognosis.<sup>152</sup> As previously noted (page 1613), the type of M-protein may be of significance as regards survival; eg, in one study, death occurred within a year of diagnosis in 57% (8/14) of patients with IgA, in 38% (5/13) of patients with Bence Jones proteinuria but without a serum M-component, and in 21% (10/48) of those with IgG.<sup>89</sup>

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# Macroglobulinemia, Heavy Chain Diseases, and Other Lymphocyte and Plasma Cell Dyscrasias

Macroglobulinemia  
Heavy Chain Diseases  
   $\gamma$ -Heavy Chain Disease  
   $\alpha$ -Heavy Chain Disease  
   $\mu$ -Heavy Chain Disease  
Amyloidosis  
"Benign" Monoclonal  
  Hypergammaglobulinemia  
Cryoglobulinemias

## Macroglobulinemia (Waldenström's Macroglobulinemia)

### Definition

The term "macroglobulinemia" includes a number of clinical conditions with a common serologic abnormality: the presence of a *monoclonal* macroglobulin which is produced by cell populations normally responsible for the synthesis of IgM globulin. Occasionally the term is used to describe a general increase in serum macroglobulins, i.e., a polyclonal elevation of serum IgM, but this practice leads to confusion and should be discouraged. Monoclonal macroglobulinemia is found under three circumstances: (1) as part of the plasma cell dyscrasia commonly identified as

*primary* or Waldenström's macroglobulinemia; (2) in association with certain malignant and progressive forms of lymphoma, nonreticular neoplasms, and, rarely, infections and inflammatory conditions (*secondary macroglobulinemia*); and (3) as a relatively stable serologic abnormality in the absence of any other identifiable disease (one form of "benign monoclonal gammopathy," page 1638).

### Etiology

A general discussion of possible etiologic factors in plasma cell dyscrasias is found in Chapters 46 and 52. A genetic predisposition has been suggested by family clusters of macroglobulinemia, as well as by the frequent occurrence of various immunoglobulin abnormalities and other immunologic abnormalities in the relatives of patients with macroglobulinemia.<sup>30,52</sup> The role of chronic inflammatory disease and nonreticular neoplasms<sup>29,41,56,62,68</sup> in the development of macroglobulinemia is the subject of much dispute.

### Clinical Features<sup>19,40 41,42,52,53,56,78</sup>

Macroglobulinemia is almost entirely a disease of the elderly, with a peak incidence



in the sixth and seventh decades. It occurs somewhat more commonly in males than in females. The disorder is frequently mild and is compatible with prolonged survival. Symptoms of vague ill health, weakness, and weight loss are common and may antedate the more serious manifestations by many years. As the disease progresses, *hepatomegaly*, *splenomegaly*, and *adenopathy* become prominent features and the clinical pattern becomes that of a lymphoma or of chronic lymphocytic leukemia. Eventually other tissues may become invaded by abnormal cells, including the lungs,<sup>58,75</sup> kidneys,<sup>52,59</sup> and central nervous system.<sup>52,54</sup>

Involvement of the *central nervous system* may be accompanied by high concentrations of macroglobulins within the cerebrospinal fluid.<sup>54</sup> Malabsorption due to the deposits of macroglobulins within the *intestinal wall* has also been reported.<sup>73</sup> In contrast to multiple myeloma, *bone pain* is not a prominent symptom and punched-out bone lesions are rarely seen.<sup>41,57,92</sup> Diffuse osteoporosis has been observed but may in many instances only reflect the patient's age.

*Renal disease* is much less common than in multiple myeloma,<sup>49,59,89,97</sup> even in the presence of Bence Jones proteinuria. The latter occurs in perhaps 10 to 30% of patients.<sup>52,59</sup> Occlusion of tubules by casts is extremely uncommon. The reason for this is unclear but may include the absence of contributing factors such as hypercalcemia and hypercalciuria. Renal amyloidosis is rare,<sup>31,59</sup> but uric acid nephropathy and interstitial infiltration with malignant cells do occur.<sup>59,97</sup> Occlusion of glomerular capillaries by subendothelial deposits of aggregated IgM proteins can be a striking finding.<sup>59</sup> Precipitation in these areas may be related to the euglobulin nature of macroglobulins, their high intrinsic viscosity, and the slow perfusion of capillaries. It is not known to what extent these deposits interfere with renal function.

*Amyloidosis* is rare and involves primarily the liver, spleen, and parenchymal organs.<sup>31,34,56,63</sup> This contrasts with multiple myeloma, in which amyloid deposits are

found primarily in the mesenchymal tissues such as muscles and the cardiovascular system (page 1634). Amyloid arthropathy has been described.<sup>34</sup>

Patients with Waldenström's macroglobulinemia frequently have decreased levels of *normal immunoglobulins* and suffer from defects in both primary and secondary antibody production.<sup>20,27,56,64,70</sup> Occasionally these defects may be severe.<sup>64</sup> The response of lymphocytes to PHA may be markedly impaired.<sup>79</sup>

### *Clinical Manifestations Caused by Abnormal Proteins*

Perhaps half the patients have clinical manifestations that are related to the specific physicochemical properties of the macroglobulins, including their high intrinsic viscosity, their ability to precipitate or gel on cooling, and their ability to participate in protein-protein interactions.

**THE HYPERVISCOSITY SYNDROME.** Viscosity is the property of fluid to resist flow. For protein solutions this resistance to flow is a function of the concentration and intrinsic viscosity of individual proteins in solution.<sup>9</sup> The intrinsic viscosity of a protein is in turn influenced by its molecular size and shape.<sup>9</sup> Since IgM molecules have a high molecular weight and an unusual shape—five projections extending outward from a central core (Chapter 7)—their intrinsic viscosity is great; macroglobulinemia, characterized by increased concentrations of such molecules, is therefore typically associated with increased serum viscosity. The serum viscosity may be further increased by the tendency of IgM molecules to aggregate,<sup>9</sup> while the viscosity of whole blood is influenced, in addition, by the tendency of protein-coated red cells to adhere to each other in rouleau formations.<sup>91</sup> These latter effects are enhanced by any decrease in velocity gradients and may account for the prominence of certain sites in the symptomatology of the hyperviscosity syndromes (see below).

While Waldenström's macroglobulinemia

is the most common cause of serum hyperviscosity, accounting for 85 to 90% of all cases,<sup>9</sup> the hyperviscosity syndrome is also noted in patients with myeloma (Chapter 52). Most frequently in myeloma the M-component is IgA, presumably because of the known tendency of IgA proteins to polymerize,<sup>9</sup> but occasionally IgG M-components lead to increased serum viscosity because of unusually high concentrations,<sup>11,52</sup> the presence of molecules with unusual (asymmetric) configurations,<sup>52</sup> or the presence of circulating aggregates of IgG M-components.<sup>6,9,13</sup> Rarely, serum hyperviscosity is noted in "connective tissue diseases" such as rheumatoid arthritis and in patients with cryoglobulins.<sup>9</sup>

The viscosity of serum is usually measured in a simple device, such as the *Ostwald viscosimeter*.<sup>28</sup> The time required for a constant volume of fluid to flow through a capillary tube is determined and is compared to the flow rate of water. The relative viscosity of normal serum (the flow time of serum divided by the flow time of water) ranges between 1.4 and 1.8.<sup>9,28</sup> It is important to make all measurements under identical conditions of pressure and temperature (usually at 37° C). A rapid screening test that uses a red cell pipette as the viscosimeter has been developed.<sup>93</sup> This test works equally well with plasma or serum and gives similar results at 37° C and at room temperature.<sup>93</sup>

The signs and symptoms of the hyperviscosity syndrome are related to the circulatory disturbances caused by the increased resistance of blood to flow. These disturbances are readily appreciated on examination of the fundi, where characteristic "link-sausage effects," consisting of alternating bulges and constrictions, are seen within the retinal veins.<sup>28,29,53</sup> Hemorrhage and exudates are also noted and visual impairment is not uncommon. Protean and everchanging neurologic manifestations, ranging from headaches, dizziness, and vertigo to somnolence, stupor, and coma, are the result of intracerebral vascular occlusions.<sup>9,28,80,94</sup> Patients may develop pareses of varying severity and Jacksonian or generalized seizures. Cere-

brovascular hemorrhage may occur secondarily. Profound, persistent deafness occurs, apparently due to thrombosis of the venous system of the inner ear.<sup>2</sup> Occlusive changes in small vessels elsewhere, due either to hyperviscosity or perhaps to cryoprecipitation, may lead to progressive peripheral neuropathies (Bing-Neel syndrome) and myelopathies.<sup>22,23,42,88</sup> Myopathies have also been described.<sup>69,87</sup> It has been suggested that elderly patients with polyneuropathies of unknown cause should always be suspected of having macroglobulinemia.<sup>89</sup>

Occasionally cardiac failure is precipitated in the elderly.<sup>9</sup> This appears to be related to hyperviscosity but may be aggravated by an increased plasma volume,<sup>53</sup> which develops in excess of that required to compensate for a decreased red cell mass<sup>53</sup> and bears a linear relationship to the relative serum viscosity.<sup>53</sup>

Hematologic complications of hyperviscosity include bleeding manifestations, which are aggravated by other effects of macroglobulins on the clotting system (see below). A relationship between erythrocyte life span and serum viscosity has been noted in some patients.<sup>18</sup>

Subjective symptoms include weakness, fatigue, and anorexia.

The threshold at which clinical symptoms occur, as well as the major target organ affected, differ from patient to patient.<sup>9</sup> However, patients with a relative serum viscosity of 2 to 4 are rarely symptomatic; many if not most patients with levels between 5 and 8 and nearly all with levels of 8 to 10 have symptoms. At relative viscosities of 10 or more all patients are symptomatic.<sup>9</sup>

The treatment of hyperviscosity syndromes will be discussed later (page 1629).

**CRYOGLOBULINS.** When a macroglobulin has the properties of a cryoglobulin (page 1640), the clinical manifestations are those of cold sensitivity, Raynaud's phenomenon, and peripheral vascular occlusion precipitated by cold. Monoclonal cold agglutinins are properly part of the macroglobulinemia spectrum but are discussed separately in Chapter 27. Occasionally, macroglobulins that are both

cryoglobulins and cold agglutinins are found.<sup>31,54</sup>

**PROTEIN-PROTEIN INTERACTIONS.** *Bleeding diathesis* results in the formation of complexes between macroglobulins and specific clotting factors such as factor VIII.<sup>16,60,61,68,69</sup> Reduced levels of factors II, V, VII, X, and XI have also been described.<sup>68</sup> Other factors contributing to the bleeding diathesis of macroglobulinemia include interference of macroglobulins with platelet function. This abnormality appears to be due to the coating of platelets by macroglobulins.<sup>45,65,69</sup> Abnormal platelet factor 3 activity has been noted.<sup>45</sup> The role of vascular damage due to increased serum viscosity, cryoprecipitation, and immune complex formation was mentioned previously.

All of these factors, in addition to thrombocytopenia (see below), result in a considerable amount of *bleeding*, particularly from the mucosae of the mouth, nose, and intestinal tract,<sup>55,61,69</sup> but bleeding from the skin may also occur. Purpura is often seen; it involves the legs predominantly. There may be prolonged bleeding at sites of minor injury. Sometimes hemorrhages involve vital areas such as the brain.

## Laboratory Findings

### The Blood

*Anemia*<sup>1,18,52</sup> is the most common presenting finding in patients with symptomatic macroglobulinemia and may become very severe. A combination of factors is responsible, including inadequate red cell production,<sup>18</sup> hemolysis,<sup>1,52</sup> and blood loss,<sup>18</sup> especially from the gastrointestinal tract. As mentioned previously, patients with an increased plasma volume due to hyperviscosity may have factitiously low hemoglobin levels.<sup>53</sup> The blood smear is usually normochromic normocytic, but rouleaux formation is often pronounced. Erythrophagocytosis has been described.<sup>44</sup>

*Leukocyte counts* are usually within the normal range, but, in some patients, neutro-

penia, as part of a general pancytopenia, has been observed. Terminally the peripheral blood may be flooded with malignant lymphoid cells that resemble those seen in the marrow (see below).

*Thrombocytopenia* is found in about half the patients suffering from a bleeding diathesis.<sup>68</sup>

*Hyperuricemia* may be present.

### Bone Marrow

It is not unusual for bone marrow punctures to result in a "dry tap." Usually this is due to the great cellularity of the marrow combined with the increased viscosity of the tissue fluid in which the cells are embedded.<sup>42</sup> *Lymphoid cells* more closely resembling lymphocytes than plasma or myeloma cells (Plate XXIII) are the predominant cell forms, but the picture is usually pleomorphic, with varying numbers of small lymphocytes showing little cytoplasm, naked nuclei, reticulum cells, and plasma cells. The last are usually too few to support a diagnosis of multiple myeloma. When the cytoplasm of lymphoid cells is sufficiently plentiful to be visible, it is often very basophilic. The periodic acid-Schiff (PAS) staining reaction frequently is positive, indicating the presence of abundant polysaccharide. The PAS staining material sometimes takes the form of globules ("grape cells"). The nucleus occasionally contains one or two nucleoli. In some cases eosinophilia has been reported, but a characteristic feature is the presence of large numbers of *basophils* and *tissue mast cells* interspersed among the other malignant cells.

### Protein Abnormalities

A markedly increased sedimentation rate and the rouleaux formation mentioned above may be the first clues to the presence of a protein abnormality. The diagnosis is confirmed by electrophoresis that reveals a *homogeneous spike* (M-component) with  $\beta$  to  $\gamma$  mobility, and by immunoelectrophoresis that establishes the IgM nature of the spike (Fig. 52-1,F). The macroglobulin contains ei-

ther  $\kappa$  or  $\lambda$  chains,  $\kappa$  chains being the more common.<sup>52</sup> Ultracentrifugation reveals a homogeneous IgM protein with a sedimentation coefficient of 19S and smaller amounts of more rapidly sedimenting components. IgM levels usually vary between 1.0 and 12 g/dl, and account for 20 to 70% of the total protein concentration.<sup>52</sup> The monoclonal IgM may be demonstrated in the cytoplasm of producing cells by immunofluorescence techniques, and is found on the surface of most proliferating lymphoid cells and on many circulating lymphocytes.<sup>72</sup>

*Urinary Bence Jones proteins* are found in up to a third of all patients and are identical to the light chains of the M-components.

The majority of macroglobulins are euglobulins and therefore give a positive reaction in the *Sia water test*,<sup>50,52,76,89</sup> especially when the protein is of  $\gamma$  mobility. In macroglobulinemic sera of  $\beta$  mobility the reaction to the *Sia water test* usually is negative.<sup>46,96</sup> Unfortunately, the test is not completely specific for macroglobulins, since certain IgG proteins also behave as euglobulins and will therefore give a positive reaction.

Some of the macroglobulins behave as *cryoglobulins* (page 1640) and yield either a whitish precipitate or a slightly turbid gel on cooling. A few have *cold agglutinin* activity (Chapter 27).

Some increase in relative serum viscosity is found in about two thirds of patients tested,<sup>52</sup> but only half of these will show clinical manifestations of the hyperviscosity syndrome (page 1626). Sometimes it is necessary to carry out viscosimetric determinations at various temperatures in order to show increased serum viscosity.<sup>87</sup>

Occasionally macroglobulins behave as *pyroglobulins* that precipitate on heating to 50 to 60° C but do not redissolve on cooling or further heating.<sup>63,66,85</sup> Most pyroglobulins are detected when serum is heated to 56° C in order to inactivate complement. Some macroglobulins have *anti-human IgG* activity.<sup>71</sup>

A significant number of sera from patients with Waldenström's macroglobulinemia con-

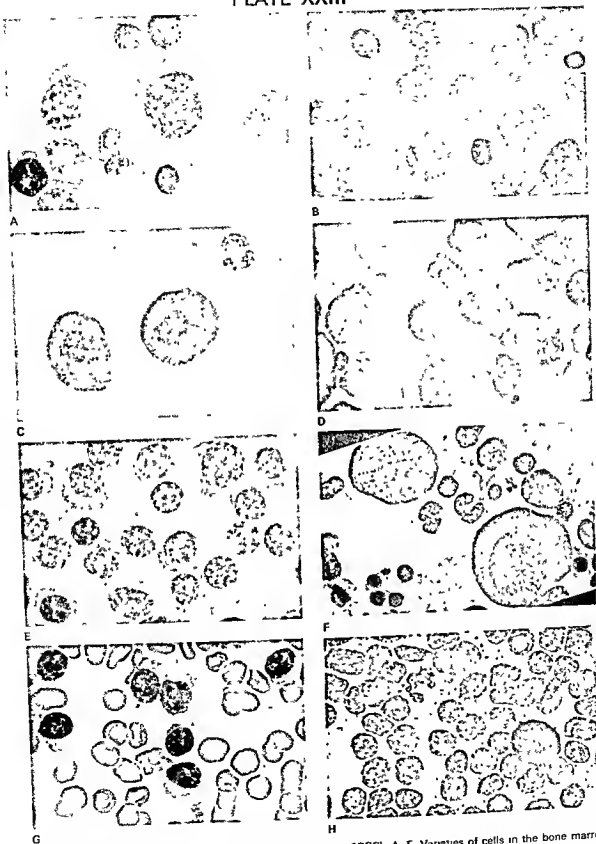
tain *low molecular weight (7S) IgM* (page 313) in addition to the typical 19S M-component.<sup>7,14,84</sup> and some produce  $\mu$  heavy chains, which apparently have lost the ability to combine with light chains.<sup>7</sup> In addition, Fab fragments, ie, fragments consisting of the amino-terminal end of the heavy chain plus its disulfide-linked light chain (Figs. 7-9 and 7-10) may sometimes be found in the urine.<sup>37</sup> A small number of macroglobulinemic patients with more than one M-component also have been described, including  $\gamma$  heavy chain fragments,<sup>43</sup> additional monoclonal IgM proteins,<sup>38,39</sup> and IgG and IgA M-components.<sup>39,57</sup>

### Differential Diagnosis

The diagnosis of macroglobulinemia is established on the basis of the characteristic serum protein abnormality and the typical changes in the blood and marrow. If there is no lymphadenopathy, hepatosplenomegaly, or obvious bone marrow abnormality, and if the concentration of the M-component does not increase over a period of years, the diagnosis of "*benign monoclonal gammopathy*" (page 1638) may be justified. Since macroglobulinemia frequently occurs in association with various malignant conditions<sup>42,52,62</sup> ("secondary macroglobulinemia"), a careful search for such conditions must always be carried out.

### Pathology<sup>24,51,52,56</sup>

Proliferation of abnormal lymphocytoid cells leads to enlargement of the spleen and lymph nodes and dense infiltrations in the periportal zones of the liver, in the kidneys, and throughout other viscera. In contrast to the findings in other lymphomas and leukemias, the lymph node architecture is largely preserved.<sup>44,56</sup> Infiltration of the small intestine and mesenteric lymph nodes is found to be associated with gastrointestinal manifestations such as diarrhea.<sup>12</sup> The central nervous system may also be infiltrated. The appearance of the lymphocytoid cells and the



Multiple myeloma and macroglobulinemia (Wright's stain,  $\times 1000$ ) A-F, Varieties of cells in the bone marrow of patients with multiple myeloma. F is from a patient with light chain myeloma. G and H are from the bone marrow of patients with Waldenström's macroglobulinemia. In G are shown a binucleated cell with nucleoli and shedding cytoplasm as well as abnormal lymphoid cells. In H, abnormal lymphoid plasma cells are seen, as well as a tissue mast cell.

bone marrow findings have been described previously (page 1627).

## Treatment

In the early stages, the disease may be stable or slowly progressive and no treatment is necessary. Later, anemia, bleeding manifestations, massive organomegaly, and symptoms related to hyperviscosity constitute the major indications for therapeutic intervention.

## Alkylating Agents

Alkylating agents are the drugs of choice in the treatment of Waldenström's macroglobulinemia. Chlorambucil,<sup>5,17,52,56</sup> melphalan,<sup>52</sup> and cyclophosphamide<sup>10,15,52</sup> have been used, and objective responses are seen in most treated patients. Such objective responses include reduction in the size of lymph nodes, liver, and spleen; decrease in M-component levels and reduction in serum viscosity to asymptomatic levels, as well as increased hemoglobin levels.

*Chlorambucil* has been used most frequently. Treatment is initiated with 6 to 8 mg daily; this dose is continued for two to four weeks. High-dose therapy (10 to 12 mg daily) carries the risk of marrow aplasia, which may be irreversible.<sup>52</sup> A maintenance dose of 2 to 6 mg daily is then instituted, and is continued indefinitely, since discontinuation leads to prompt relapse that may be difficult to control by reinstitution of drug therapy.<sup>56</sup> Some patients have been maintained on chlorambucil for many years, apparently in good remission. It is important to monitor marrow function by means of frequent blood counts during therapy in order to forestall unnecessary marrow toxicity.

*Cyclophosphamide* has been used in doses of 50 to 150 mg daily with equally good results.<sup>10,15,52</sup> High-dose intermittent regimens have not yet been evaluated in macroglobulinemia. Steroids are not generally

given, but have been used, especially in patients suffering from leukopenia and thrombocytopenia.<sup>73</sup>

## Plasmapheresis

All manifestations of the *hyperviscosity syndrome* respond to plasmapheresis.<sup>9,28,33,52,80,83</sup> The effectiveness of this procedure is due to two factors. Approximately 80% of all IgM is confined to the intravascular space<sup>28</sup> and the viscosity increment becomes progressively greater with each unit increase in IgM concentration.<sup>9,26</sup> Thus, at higher macroglobulin levels, relatively small reductions of serum macroglobulin concentrations (15 to 20%) may reduce the relative viscosity by 50 to 100%.<sup>9</sup> Often, however, it is necessary to remove at least half the plasma volume or more, in order to lower the serum viscosity significantly. As a guide to therapy, viscosity measurements should be made both before and after plasmapheresis. Once control of symptoms has been achieved, plasmapheresis must be repeated, since the effects of this procedure are temporary. Generally, 2 to 4 units of plasma have to be removed every week or two.<sup>9</sup> Temporary benefit has also been reported with the use of penicillamine or other chelating agents, presumably because these drugs can dissociate macroglobulin aggregates by reducing disulfide bonds.<sup>8,48</sup> Of greatest long-range promise is the use of alkylating agents (see above) and/or corticosteroids which, if effective, may lead to a prolonged reduction of serum viscosity to asymptomatic levels.

## Prognosis

Prognosis appears to be closely linked to response to therapy. In one study,<sup>52</sup> patients who responded to therapy had an average survival of four years from the time of diagnosis, whereas unresponsive patients lived only two years. Others have quoted overall average life expectancies of 38 to 40 months from the time of the first complaints.<sup>42</sup> Much

individual variation is seen, however, since the process is quite malignant in some and comparatively benign in others, with survivals of a decade or longer.<sup>19</sup>

## Heavy Chain Diseases

### Definition

Heavy chain diseases (HCD) are lymphocyte dyscrasias that are characterized by the malignant proliferation of immunoglobulin-producing cells and the concomitant synthesis of monoclonal proteins (M-components, page 1600), which have the immunochemical characteristics of incomplete heavy (H) chains. The abnormal cells are predominantly lymphocytes or plasma cells. The clinical manifestations of these diseases more closely resemble those of lymphomas than those of multiple myeloma. The first patient was described by Franklin in 1963<sup>117</sup> and since then an increasing number of cases have been reported.<sup>113</sup>

On the basis of structural differences within the heavy chain, five major immunoglobulin classes are recognized (heavy chain designations in brackets): IgG ( $\gamma$ ), IgA ( $\alpha$ ), IgM ( $\mu$ ), IgD ( $\delta$ ), and IgE ( $\epsilon$ ) (see Chapter 7 for details). So far only  $\gamma$ -,  $\alpha$ -, and  $\mu$ -heavy chain diseases have been reported;  $\delta$ - and  $\epsilon$ -heavy chain diseases have not yet been encountered. Since the clinical manifestations vary considerably, depending on the heavy chain class,  $\gamma$ -,  $\alpha$ -, and  $\mu$ -heavy chain diseases will be discussed separately.

### $\gamma$ -Heavy Chain Disease ( $\gamma$ -HCD)<sup>102,105,109,113,117,127,</sup>

145, 147, 148, 150, 152

### Clinical Features

During the 10 years following Franklin's initial description, approximately 30 cases of  $\gamma$ -HCD have come to light.<sup>113</sup> The disease has been reported in Caucasians, Negroes, and Orientals. There is a slight male-over-female preponderance. The age incidence has ranged between 18 and 76 years, but only four of 30 patients were under the age of 40.

The initial clinical manifestations may develop rapidly but more frequently are gradual in onset. *Lymphadenopathy*, *anemia*, and *fever* are the most common presenting features, but many patients also have *hepatosplenomegaly*. As the disease progresses, lymphadenopathy and hepatosplenomegaly become even more frequent and pronounced. Cervical, axillary, intrathoracic, and intra-abdominal nodes are most frequently involved,<sup>102</sup> but, occasionally, peripheral lymphadenopathy is absent.<sup>102</sup> *Palatal erythema and edema*, similar to those of infectious mononucleosis, are often seen and may give rise to respiratory difficulties. The palatal findings are probably due to involvement of the nodes of Waldeyer's ring. Recurrent bacterial *infections* are common and are the most frequent cause of death. *Bony lesions* similar to those of multiple myeloma appear to be rare, but have been reported.<sup>102,113</sup>

As in other plasma cell dyscrasias, a good number of patients with  $\gamma$ -HCD appear to have *associated diseases* with autoimmune features, such as lupus erythematosus, rheumatoid arthritis, Sjögren's syndrome, pulmonary fibrosis, myasthenia gravis, thyroiditis, and hemolytic anemia.<sup>113,123,138,150</sup> In addition, an antecedent history of chronic tuberculosis and cholecystitis has been reported.<sup>113</sup> In other patients, polyclonal hypergammaglobulinemia preceded the discovery of the M-component.<sup>109,117</sup> It is not known whether these conditions and HCD are etiologically related, but the possibility must be considered. Viral particles were seen in the tissues of one subject.<sup>117</sup>

### Laboratory Findings<sup>113</sup>

#### *The Blood*

Anemia is a universal finding. About two thirds of the patients have leukopenia that is usually due to granulocytopenia, and, in an equal number, atypical lymphocytes or plasma cells are seen in the blood smear. In patients with terminal illness the latter cells may occasionally be so frequent as to lead to the diagnosis of "plasma cell leukemia"

(Chapter 52). Eosinophilia was noted in two of the initial patients, but this finding has not been prominent in most reports.<sup>102</sup> About half the patients have thrombocytopenia. The ESR may be elevated. Hyperuricemia may be present.

### **Bone Marrow**

The bone marrow specimen is occasionally completely normal but usually shows an increase in plasma cells and/or lymphocytes and reticulum cells. Eosinophilia has been noted in combination with the other findings or, occasionally, as the sole abnormality. Biopsy or necropsy examination of lymph nodes and other involved organs has shown a similarly pleomorphic picture. An inaccurate pathologic diagnosis of Hodgkin's disease or a "granulomatous condition" is not uncommon in the early stages of the disease. Amyloid deposits have been discovered occasionally on postmortem examination.

### **Serology<sup>102,113</sup>**

The diagnosis of  $\gamma$ -HCD is generally based on the discovery in the serum and/or urine of an abnormal protein migrating in the  $\gamma$  to  $\beta$  region in conventional electrophoresis. On immunoelectrophoresis, these proteins are reactive with anti- $\gamma$  sera but not with antisera to Fab fragments (Chapter 7) or L (light) chains. In about half the patients the serum component is present in amounts greater than 2 g/dl; in the remainder the concentration is less. In most instances the spike is quite broad and heterogeneous and is accompanied by depressed normal gamma-globulin levels.<sup>102</sup> A poor antibody response to bacterial antigens and depressed cellular immunity have been found in a few patients.<sup>102</sup> A Bence Jones protein, macroglobulin, and H-chain fragment were discovered concomitantly in one patient.<sup>122</sup>

### **Protein Abnormalities**

Structural studies of the abnormal proteins have revealed sedimentation coefficients of 3.5 to 4.0S and molecular weights of 45,000 to 80,000. The proteins are unusually rich in carbohydrates. Most proteins belong to the  $\gamma_1$  subclass (see page 309 for a description of IgG subclasses), but the other three sub-

classes ( $\gamma_2$ ,  $\gamma_3$ ,  $\gamma_4$ ) have also been seen.<sup>102,113</sup> The abnormal proteins are H-chain fragments of several structural types<sup>113</sup> (Fig. 53-1). There may be (1) massive deletion of Fd variable and Fd constant regions, with resumption of normal synthesis at residue 216 (Glu)<sup>114,131</sup>; (2) deletion of the hinge region only (residues 216 to 232)<sup>111</sup>; (3) deletions of the Fd variable, Fd constant, and hinge regions, with resumption of synthesis at a point beyond the hinge region<sup>105,115</sup>; and, finally, (4) proteins that look like enzymatic degradation products derived from longer H chains, whose sequences most commonly start in the hinge region.<sup>130,144</sup> The mechanisms responsible for the structural deletions of  $\gamma$ -chain fragments are not known.

Sometimes the protein abnormalities are more complex; thus, the serum of one patient contained a monoclonal IgG $\lambda$  protein with deletions in both heavy and light chains (est. mol. wt. 110,000) and free  $\gamma$  chain fragments; deleted  $\lambda$  chains (est. mol. wt. 15,000) were found in the urine. The deletions in both heavy and light chains occurred in their respective variable regions and appeared to be comparable.<sup>119</sup>

### **Treatment**

Treatment is often unsatisfactory. Local radiation to spleen and lymph nodes appears to be rapidly effective in reducing the size of the involved organs,<sup>102,117,127,136</sup> but usually the effect is transient. Prolonged beneficial effects are occasionally observed.<sup>136</sup> Alkylating agents have not been beneficial in doses and treatment schedules used to date,<sup>102,113</sup> except for transient responses in occasional patients.<sup>109,127</sup> A good response to prednisolone alone<sup>145</sup> and to vinblastine<sup>108</sup> has been observed in single patients. Combination drug therapy (nitrogen mustard, vincristine, prednisone, and procarbazine) appears to be promising.<sup>106</sup>

### **Prognosis**

Thus far, prognosis has been unfavorable, many patients having died within a matter of months from infections or other effects of the malignant condition, but some have survived for five years and more.<sup>113,149</sup>



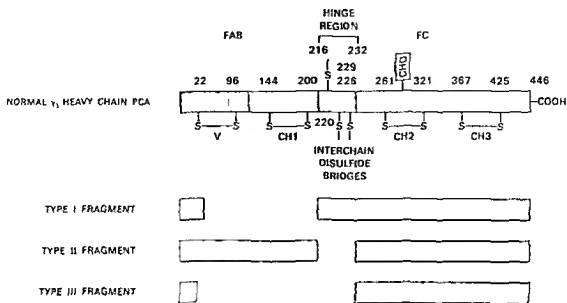


Fig. 53-1 Schematic representation of some deletion patterns currently recognized in  $\gamma$ -heavy chain disease. Fragments (below) are compared to normal  $\gamma_1$  heavy chain (above). Variable region (V), heavily shaded; hinge region, lightly shaded. Numbers refer to amino acid residues. CH 1, 2, 3 = constant regions. See Chapter 7 and Figure 7-9 for details of immunoglobulin structure. Deletions of heavy chain fragments indicated in light lines. See text for details of fragment structure. (Adapted from Frangione and Franklin<sup>114</sup>)

### $\alpha$ -Heavy Chain Disease ( $\alpha$ -HCD)<sup>113,133,139,141</sup>

#### Clinical Features

$\alpha$ -HCD was first described by Seligmann in 1968<sup>140</sup> and seems to be the most common form of HCD.<sup>133,141</sup> In contrast to  $\gamma$ -HCD,  $\alpha$ -HCD has a predilection for younger age groups, including children. The areas of involvement correspond to the main sites of secretory IgA production, particularly the gut, or occasionally, the respiratory tract.<sup>113,133,141</sup> Infiltration of the lamina propria of the intestine and of abdominal lymph nodes with plasma cells, lymphocytes, and reticulum cells is a characteristic feature of the disease and gives rise to severe malabsorption, diarrhea, and hypocalcemia. In most cases the infiltrate is confined to the lamina propria of the small intestine, but occasionally involvement of the rectum or stomach and spread to the bone marrow and even to the postnasal space are seen.<sup>133</sup>

$\alpha$ -HCD is most commonly associated with a condition previously identified as "Mediterranean lymphoma" because the first described

patients were non-Ashkenazi Jews or Israeli Arabs.<sup>134,139,140</sup> The disease does not appear to be restricted to these population groups, however.<sup>126,128</sup>

Whether  $\alpha$ -HCD is malignant from the beginning or starts as a benign hyperplastic process, perhaps in response to a high degree of intestinal infection,<sup>133</sup> is unknown. Some patients eventually develop reticulum cell sarcoma.<sup>133</sup>  $\alpha$ -HCD must be differentiated from nodular intestinal lymphomas (Chapter 51).

#### Laboratory Features

Since  $\alpha$ -HCD does not usually involve the marrow, changes in the blood are invariably due to other complications, such as malabsorption or bleeding. The serum alkaline phosphatase (intestinal isoenzyme) may be elevated.<sup>133</sup> A defect in systemic immunity has been demonstrated in two patients,<sup>133</sup> but local intestinal immunity has not been studied.

On electrophoresis  $\alpha$ -heavy chains usually

migrate in a broad peak with  $\beta$  to  $\alpha_2$  mobility. This is due to their tendency to polymerize, which may also explain the relatively low excretion of  $\alpha$ -chains in the urine, in comparison to  $\gamma$ -chains.  $\alpha$ -Chains may also be recovered from jejunal juice,<sup>126</sup> and this is sometimes the preferred method of diagnosis. On immunoelectrophoresis the protein reacts with anti- $\alpha$  sera but not with anti-light chain sera. Unfortunately many intact IgA myeloma proteins also do not react with antisera to light chains<sup>113</sup> and this, in addition to the low concentration of  $\alpha$ -chains in the urine, frequently makes diagnosis difficult. Only the  $\alpha_1$  subclass (Chapter 7) has been identified so far, even though most normal secretory IgA is of the subclass  $\alpha_2$ .<sup>133</sup>

A small bowel biopsy specimen will show the characteristic cellular infiltrates described above. The liver, spleen, and peripheral lymph nodes usually are not involved.

### Treatment

Therapy is usually unsatisfactory, but good responses leading to prolonged remissions have been reported with radiotherapy and prednisone,<sup>126</sup> as well as with cyclophosphamide.<sup>153</sup> Complete remissions of the disease after treatment with antibiotics alone have also been recorded.<sup>133,135</sup>

### $\mu$ -Heavy Chain Disease ( $\mu$ -HCD)<sup>101,107,112,113,124</sup>

#### Clinical Features

This is the rarest of the heavy chain diseases and only a few patients have been described.<sup>101,103,112,120,124</sup> Most but not all<sup>101a,102a</sup> have had long-standing chronic lymphocytic leukemia (CLL)<sup>107,113</sup> or non-Hodgkin's lymphoma.<sup>118,120</sup> The disease is manifested primarily by visceral organ involvement including hepatomegaly, splenomegaly, and enlarged abdominal nodes with little, if any, peripheral lymphadenopathy. One patient had amyloidosis and two had suffered pathologic fractures. Another patient developed a  $\mu$ -chain fragment in association with an ill-defined lymphoma-like disorder characterized by an indurated ulcerating lesion over the left pa-

rotid gland and massive splenomegaly.<sup>120</sup> In most instances, the protein was discovered in the later stages of the disease process and it is not known whether it was present from the beginning or whether its discovery is a bad prognostic sign. Prolonged survival has been observed after the diagnosis of  $\mu$ -chain disease was made.<sup>118,124</sup>

### Laboratory Features

In patients with CLL the main hematologic findings are those of the underlying disease (Chapter 49), except that strikingly vacuolated plasma cells are found in the marrow.<sup>113</sup>

The routine electrophoretic pattern does not reveal  $\mu$ -chain protein, but marked hypogammaglobulinemia usually is seen. Immunoelectrophoresis reveals a rapidly migrating protein reactive with anti- $\mu$  sera but not with antisera to light chains. The protein is not found in the urine, perhaps because of polymerization of the fragments,<sup>116</sup> but the serum protein appears to exist predominantly in the monomeric form.<sup>120</sup> Some patients had heavy Bence Jones proteinuria in addition to the heavy chain fragment,<sup>113,118</sup> and it has been suggested that an abnormality in H-chain synthesis, perhaps a deletion, may preclude normal H- and L-chain association in these instances.<sup>103,154</sup> Another patient produced, in addition, a monoclonal IgA protein.<sup>120</sup>

Therapy is directed at the underlying disease (Chapter 55).

## Amyloidosis

### Definition

Amyloidosis is a disease characterized by the presence of a homogeneous, eosinophilic material in various tissues throughout the body. The hallmark of these deposits is the amyloid fibril, which is readily identified by its green polarization birefringence following staining with Congo red, as well as by a unique x-ray diffraction pattern. The deposition of amyloid proteins in vital structures

is responsible for the replacement and destruction of the latter and, often, the eventual death of the patient. The term "amyloid" was introduced by Virchow because of the apparent affinity of the material for iodine, which suggested a resemblance to starch. Carbohydrates are now known to be a minor and relatively unimportant component of amyloid.<sup>200</sup>

### Classification (Table 53-1)

Amyloidosis was first described in association with chronic sepsis such as tuberculosis, and this form is now identified as "secondary amyloidosis." It soon became evident that material indistinguishable from this type of amyloid could also be identified in the myocardium and other tissues of certain individuals in the absence of chronic infection. Such patients are said to suffer from *primary amyloidosis*. Severe forms of this condition are

much less common than cases of secondary amyloidosis, but clinically undetectable deposits of amyloid are quite often seen in the elderly (*senile amyloidosis*).<sup>172,177,207,214,252,255</sup>

Amyloidosis is also found in association with various plasma cell dyscrasias, especially multiple myeloma, and this form of the disease most closely resembles primary amyloidosis. Familial forms of amyloidosis also have been described and often affect specific organ systems, resulting in polyneuropathy, nephropathy, or cardiopathy.

In the past much has been made of the differential organ involvement in primary and secondary amyloidosis. Thus, it was stressed that primary amyloidosis and amyloidosis occurring with multiple myeloma typically involved mesodermal tissues such as smooth and skeletal muscle and the cardiovascular system, whereas secondary amyloidosis involved primarily the liver, spleen, and kidneys. A great deal of overlap is seen, however.<sup>171,177,217</sup> and while clinical differences between primary and secondary amyloidosis are demonstrable in large series, they probably are no greater than the differences between individual cases within either category.<sup>217</sup> Existing differences in the deposition of amyloid may be related to differences in the precursor protein, and in its rate of synthesis or catabolism, or in the anatomic relationship between the cell producing the precursor protein and the cell producing the fibril from the precursor (see below).<sup>200</sup>

### The Nature of Amyloid<sup>191,199,209</sup>

The first chemical analysis of amyloid was made in 1859 and suggested that the material was a protein.<sup>189</sup> Since then there has been much controversy about the origin and chemical composition of amyloid.<sup>213</sup> A link between amyloidosis and the immune system had been suspected for many years because of the frequent association of amyloidosis with plasma cell dyscrasias,<sup>220,227,229</sup> the demonstration of an increased number of plasma cells in the marrow of some patients with primary amyloidosis,<sup>162</sup> and the association between chronic antigenic stimulation

**Table 53-1. Amyloidosis—Classification**

I	Primary amyloidosis
II	Amyloidosis with plasma cell dyscrasias
III	Secondary amyloidosis
A	Chronic infections
	Tuberculosis
	Osteomyelitis
	Leprosy
	Syphilis
	Bronchiectasis
	Reiter's syndrome
	Whipple's disease
B	Autoimmune diseases
	Rheumatoid arthritis and others
	Ulcerative colitis
	Regional enteritis
C	Neoplasms
	Hodgkin's disease
	Renal cell carcinoma
	Medullary carcinoma of the thyroid
IV	Heredofamilial amyloidoses
A	Familial amyloid polyneuropathy
B	Familial amyloid cardiopathy
C	Amyloid nephropathy of familial Mediterranean fever
D	Familial amyloid nephropathy
E	Familial cutaneous amyloid
F	Familial medullary thyroid carcinoma producing amyloid

and so-called "secondary" amyloidosis.<sup>206,239,256</sup> In addition, immunofluorescence and other immunochemical studies occasionally revealed immunoglobulins or their component parts in amyloid.<sup>243</sup> However, it remained for electron microscopic investigations to reveal a fibrillar component of distinctive appearance<sup>178,179,236,245,246</sup> which was shown to be the structure responsible for the Congo red staining of amyloid and its birefringence under polarized light. X-ray crystallographic analysis subsequently demonstrated that these fibrils are composed of polypeptide chains arranged in an antiparallel conformation and a  $\beta$ -pleated sheet structure.<sup>182</sup> Eventually, ways were found to solubilize and purify these proteins<sup>193,195</sup> and this resulted in their physical, chemical, and immunochemical characterization.<sup>195,205</sup>

The major protein components of amyloid fibrils may be of immunoglobulin or non-immunoglobulin origin.

**AMYLOID OF IMMUNOGLOBULIN ORIGIN.** *Amyloid fibrils of immunoglobulin origin* are most frequently associated with plasma cell dyscrasias or with primary amyloidosis, but have also been found in patients with secondary amyloidosis.<sup>199,200,222</sup> The immunoglobulin nature of these fibrils has been proved conclusively by amino acid sequence analysis,<sup>197,249</sup> which revealed structures similar to those of Bence Jones proteins.<sup>200,249</sup> In addition, the sequence of amyloid fibrils derived from a given individual was found to be homogeneous, thereby indicating the probable origin of these fibrils from a single clone of immunoglobulin synthesizing cells. The molecular weights of fibril proteins range from 5,000 to 18,000,<sup>195,200,205</sup> whereas those of intact light chains are 22,500. Thus, in most patients with amyloidosis only fragments of light chains are contained in amyloid deposits and most of these appear to be derived from the amino terminal or variable portion (page 308) of the light chain, i.e., the portion involved in antigen binding. Since most antisera against light chains react with antigens in the constant (partially missing) region (page 308) of the molecule or with idiotypic determinants of the variable region, it is not

surprising that many workers were unable to confirm the immunoglobulin nature of amyloid by immunofluorescence and other techniques.<sup>200</sup> Occasionally, fibrils may contain either an intact light chain or a combination of intact light chains and light chain fragments.<sup>200</sup>

The source of amyloid fibrils is not always clear.<sup>199</sup> (1) They may be derived from circulating M-components such as Bence Jones proteins or from whole immunoglobulin molecules. This is almost certainly the case in amyloidosis associated with plasma cell dyscrasias. In this connection it is of interest that amyloid fibrils may be produced by proteolytic digestion of monoclonal light chains,<sup>196,218</sup> while in other situations Bence Jones proteins can be converted directly to amyloid fibrils by thermal treatment.<sup>199</sup> (2) Antigen-antibody complexes may be catabolized by macrophages and fibrils may be deposited in macrophage-rich organs, such as the liver and spleen, producing a distribution most commonly associated with secondary amyloidosis.<sup>199</sup> (3) Deletions in the light chain gene might result in fragments analogous to those seen in heavy chain disease (page 1631); or (4) there may be separate and disproportionate synthesis of variable and constant regions of light chains<sup>196,242</sup> leading to fibril formation.

**AMYLOID OF NON-IMMUNOGLOBULIN ORIGIN.** In some patients the amyloid deposits are derived from proteins that have no resemblance to immunoglobulins.<sup>165,183,196,199,215</sup>

This material has been referred to as A-component, nonimmunoglobulin acid-soluble fraction (ASF) or "amyloid of unknown origin" (AUO),<sup>200,214</sup> since the responsible protein has not yet been identified. These amyloid fibrils may be derived from proteins associated with immunoglobulins such as the J-piece, the secretory piece, or complement components (Chapter 7).<sup>200</sup> AUO is usually found in patients suffering from the secondary form of amyloidosis and may originate from a larger protein by proteolytic digestion.<sup>200</sup> While the structure of immunoglobulin-derived amyloid fibrils tends to vary from patient to patient, all

amyloid fibrils of unknown origin appear to have an almost identical N-terminal sequence.<sup>200,214,215</sup> A component which cross reacts with ASF has also been found in the sera of a small number (7%) of normal individuals and in the sera of a larger number (50 to 80%) of patients who suffer from conditions known to be associated with amyloidosis.<sup>215</sup> Sometimes smaller quantities of immunoglobulin proteins have been found in addition to the AUO.<sup>168,200</sup>

**OTHER FORMS OF AMYLOID.** Possibly a third form of amyloid has been identified in association with various endocrine tumors such as medullary carcinoma of the thyroid gland and insulinomas.<sup>221,232</sup>

**OTHER COMPONENTS OF AMYLOID.** Other components identified within amyloid deposits include fibrinogen, lipids, lipoprotein, complement components (especially in the secondary form), and polysaccharides.<sup>200</sup> Of special interest is the presence of small (8-nm) doughnut-like, pentagonal structures composed of five globular subunits surrounding a central cavity.<sup>167,192</sup> This morphologically distinct unit has also been shown to aggregate into rods with a 4-nm periodicity<sup>192</sup> and appears to consist of glycoproteins that are unrelated to the protein component of fibrils.<sup>191</sup> Antibodies against the pentagonal component cross-react with normal serum components migrating in the  $\alpha_1$  region.<sup>200</sup> By immunofluorescence techniques the material has been identified in all amyloid deposits studied so far.<sup>176</sup>

### Incidence

The prevalence of amyloidosis in the population at large is not known. Routine autopsy data from general hospitals show an incidence of about 0.5%,<sup>177</sup> but these data are probably falsely low because special stains are not routinely employed. In leprosy, chronic tuberculosis, and similar conditions, the incidence may be as high as 50%, and similarly high figures have been quoted for hereditary forms of amyloidosis associated with familial Mediterranean fever.<sup>177</sup> In selected populations of old people, such as those

suffering from senile dementia, the incidence may approximate 90%.<sup>244</sup>

### Clinical Features

The clinical manifestations of amyloidosis are determined by the distribution of tissue infiltrates. *Renal involvement* is the most common and potentially the most serious manifestation of amyloidosis and is the major cause of death in most series.<sup>169,171,181,217,230</sup> It is equally frequent in primary and secondary amyloidosis<sup>171,217</sup> and occurs as the major manifestation of the amyloid associated with familial Mediterranean fever.<sup>171</sup> The lower urinary tract and other urogenital organs also may be involved. Occasionally renal amyloidosis may be completely asymptomatic, but most patients exhibit proteinuria, which may be massive, and perhaps 60% of all patients eventually become nephrotic.<sup>177</sup> In addition to proteinuria, hyposthenuria and persistent hematuria may be found. Azotemia is a late manifestation of renal amyloidosis.

*Cardiac involvement* occurs in most patients (80 to 90%) with primary amyloidosis and in more than half of those with secondary amyloidosis.<sup>177</sup> It may also occur as a familial condition.<sup>187</sup> Amyloid infiltration of the heart typically results in enlargement without changes in the intracardiac volume, as occurs in heart failure from other causes. The stiff, thickened myocardium may yield cardiac catheterization data which are erroneously attributed to constrictive pericarditis. In addition to cardiac failure, manifestations of amyloid heart disease include conduction disturbances, arrhythmias, and symptoms of coronary artery insufficiency. The electrocardiogram often shows low voltage in the QRS complex. Heart failure due to amyloidosis may be quite intractable; in addition, some patients are quite sensitive to digitalis and may develop serious as well as lethal arrhythmias.<sup>175,233</sup>

*Gastrointestinal involvement* is common in all forms of amyloidosis. It occurs at all levels of the gastrointestinal tract and may lead to obstruction, diarrhea,<sup>188</sup> hemorrhage,<sup>169,209</sup> and secondary manifestations such as vitamin

$B_{12}$  deficiency,<sup>169,170</sup> malabsorption,<sup>169,188</sup> and protein-losing enteropathy.<sup>209,237</sup> Liver involvement occurs in virtually all patients with secondary amyloidosis and in most patients with primary amyloidosis.<sup>171,216</sup> Livers weighing 4 to 9 kg have been reported, but there is often little impairment of hepatic function and laboratory studies may reveal little or no abnormality.<sup>177</sup> The BSP test and serum alkaline phosphatase levels are the most useful laboratory procedures for assessing the function of amyloid livers.

The respiratory tract may be involved in about 20% of secondary amyloidosis patients and in the majority of patients with primary amyloidosis. Amyloid may involve the oral mucosa, tongue, gingiva, sinuses, and the area around the larynx, as well as the trachea, bronchi, and lungs.<sup>168,177,235</sup> The symptoms depend on the anatomic location of the amyloid.

Amyloidosis of the nervous system may result in peripheral neuropathy with sensory disturbances, painless ulcers, and weakness of the legs. The autonomic nervous system may also be affected, with sphincter disturbances, impotence, dyshidrosis, or orthostatic hypotension.<sup>177,226</sup>

The "carpal tunnel syndrome" with medial nerve compression and arterial insufficiency of the hands may result from infiltration of the carpal ligaments. Amyloid deposits may also involve joints in which they frequently produce symptoms that mimic those of rheumatoid arthritis.<sup>202,234</sup> Amyloidosis of various endocrine organs occurs occasionally. Virtually all other sites of the body may be involved with amyloid, in the form of either small infiltrates or large tumors.

Amyloidosis of the skin<sup>177,221</sup> is characterized by hyaline plaques which occur most commonly in skin folds such as the inguinal area. When the small vessels of the skin and mucous membranes are involved as well, diffuse purpura may be seen. Often profuse bleeding occurs after minor surgical procedures such as those performed for securing gingival or skin biopsy specimens. When amyloidosis occurs in association with plasma cell dyscrasias, defects in hemostasis due to

the latter (page 1610) may further aggravate the bleeding tendency. A few cases of amyloidosis with an acquired factor X deficiency have been described.<sup>166,253</sup>

Some patients with an underlying plasma cell dyscrasia suffer from a particularly severe form of dermal amyloidosis referred to as lichen myxedematosus or papular mucinosis.<sup>223,228,241</sup> In some cases a uniquely basic cationic M-component of the IgG class has been identified<sup>208</sup>; this has a strong affinity for constituents of normal skin.<sup>223,228</sup> It is not known whether these M-components have autoantibody activity towards normal skin or whether the interaction is a nonspecific one. In most patients with lichen myxedematosus, the skin disease becomes progressively worse and may on occasion appear as a prelude to multiple myeloma.<sup>233</sup> Resolution of skin lesions has been observed following successful therapy of the associated plasma cell dyscrasia with melphalan.<sup>185</sup>

### Laboratory Features

Because amyloid infiltrates may involve many organ systems a great variety of hematologic and biochemical abnormalities may be produced. Some of these are the result of underlying diseases such as chronic sepsis or a plasma cell dyscrasia. A diligent search must be made for the latter in all patients. Such studies must include electrophoretic examination of a concentrated urine specimen, since the presence of light chains in the urine is often the only demonstrable protein abnormality. An associated factor X deficiency should be looked for in patients with hemorrhagic tendencies.<sup>166,253</sup> Diagnostic features of plasma cell dyscrasias have been discussed previously (page 1614).

### Diagnosis

The diagnosis of amyloidosis is best confirmed by biopsy. Gingival biopsy is useful in more than half of all the patients, but rectal biopsy may be even more rewarding. Skin biopsies may provide positive evidence of the disease even in the absence of visible lesions.

Renal and hepatic biopsies carry a greater risk of serious hemorrhage, and rupture of the liver has been reported. Tissue sections should be stained with Congo red and examined under regular and polarized light. The appearance of areas exhibiting a characteristic green color together with dichroism constitutes almost specific histologic evidence for the presence of amyloid. Definitive confirmation requires electron microscopy.

The Congo red test, which was based on the increased rate of disappearance of this dye from the circulation of patients suffering from amyloidosis,<sup>177</sup> has been abandoned because of the occasional occurrence of severe toxic reactions and the greater specificity of histologic methods.

### Prognosis and Therapy

Because such a variety of clinical presentations are seen in amyloidosis, it is impossible to quote accurate figures regarding prognosis. In many series the prognosis is weighted because the disease was diagnosed late or at postmortem examination. In other series the underlying disease is the immediate cause of death. Nevertheless, one- to four-year survivals after diagnosis have been quoted in most larger series.<sup>177</sup> In rare instances of "secondary amyloidosis," regression of amyloid deposits has been noted after successful therapy of the underlying disease. Such patients have included occasional ones with chronic infections such as tuberculosis or osteomyelitis,<sup>212,250,251</sup> and occasionally those with tumors such as renal carcinoma.<sup>231</sup>

With the exception of the favorable effects of therapy in these few patients, no specific and efficacious form of treatment is currently available. In some patients with amyloidosis associated with plasma cell dyscrasias, attempts have been made to treat both conditions with cyclophosphamide or melphalan, but while it has been possible to lower the concentration of M-components in some patients, the clinical results have been disappointing.<sup>229,234</sup> Rare exceptions have been noted.<sup>210</sup> Attempts have also been made to treat primary amyloidosis patients with cyto-

toxic drugs, but with equally unsatisfactory results.<sup>164</sup> Steroids are of no use in treating patients with any form of amyloidosis, including amyloid nephrosis.<sup>177</sup>

## "Benign" Monoclonal Hypergammaglobulinemia

### Definition

When electrophoretically homogeneous immunoglobulins appear in the *absence* of malignant plasma cell dyscrasias or infection, the condition is referred to as "benign monoclonal hypergammaglobulinemia" (BMH) or "benign monoclonal gammopathy," a term initially proposed by Waldenström.<sup>292</sup> M-Components occurring in association with lymphoreticular malignant disorders such as lymphomas and leukemias are excluded from this definition, but those found in association with other forms of malignant conditions are not. In these cases the term "benign" refers to the behavior of the cells producing the monoclonal gamma globulin, and not to those of the associated tumor. Sometimes, only after prolonged observation is it possible to ascertain whether a given instance of monoclonal gammopathy is benign or malignant. However, such manifestations as bone lesions or anemia strongly suggest a malignant state.

Benign M-components may belong to any of the known immunoglobulin classes and are immunochemically indistinguishable from similar proteins produced by malignant plasma cell dyscrasias.<sup>261,272,276,297</sup> Benign Bence Jones proteinuria also has been described.<sup>290</sup>

### Incidence

Benign monoclonal hypergammaglobulinemia is a surprisingly common condition and its incidence appears to increase with age. In one study of nearly 7000 sera the incidence was found to be 1% in subjects more than 25 years of age<sup>261</sup> and 3% in those over the age of 70.<sup>274</sup> Others have reported an incidence of 0.1 to 0.3% in healthy blood donors.<sup>272</sup> Thus a large number of individuals,

many apparently normal, carry benign M-components and since the yearly incidence of myeloma is only two to four per 100,000 (Chapter 52), most of these individuals will never develop a malignant plasma cell dyscrasia.

## Etiology

It is obvious from Table 53-2 that benign monoclonal hypergammaglobulinemia occurs under a great number of circumstances which defy unifying concepts of etiology and pathogenesis. Several observations are, however, of interest:

1. It is quite characteristic of BMH that the concentration of the monoclonal protein remains constant over many years.<sup>263,266</sup> Only a small number of such individuals show a very slow and almost imperceptible rise in the concentration of the M-component and eventually develop multiple myeloma or a related plasma cell dyscrasia, often after 10 to 20 years of observation.<sup>279,283</sup> Thus the question remains whether BMH represents a "premyeloma state"<sup>279</sup> and whether all patients with BMH would eventually develop a malignant plasma cell dyscrasia, provided they lived long enough.

2. Since some pathologic M-components appear to have specific antibody activity (page 1605) it is possible that BMH may represent a monoclonal response to antigens encountered during the course of infections or tumor growth. Attempts to establish specificity of benign monoclonal proteins for infectious agents have failed so far,<sup>295</sup> but, in a few instances, tumors were found to be infiltrated with plasma cells containing the patient's own M-component and sometimes M-components could also be identified on the surface of tumor cells.<sup>295</sup> Thus plasma cells infiltrating the tumor may actually be the source of M-components and may represent an immune response against the tumor. In no recorded case, however, has the M-component disappeared after the removal of the tumor,<sup>293,295</sup> with the possible exception of one patient with a benign parathyroid adenoma.<sup>267</sup> It is, of course, equally possible that

**Table 53-2. Conditions That Have Been Associated with Benign Monoclonal Hypergammaglobulinemia**

<b>I Chronic sepsis</b>	
Tuberculosis	<sup>275, 276, 282, 285</sup>
Osteomyelitis	<sup>275</sup>
Pyelonephritis	<sup>285, 295</sup>
Cytomegalovirus infection	<sup>294</sup>
<b>II "Autoimmune" diseases</b>	
Rheumatoid arthritis	<sup>275, 276, 282</sup>
Penarteritis nodosa	<sup>275</sup>
Scleroderma	<sup>282</sup>
Pemphigus	<sup>282</sup>
Necrotizing vasculitis	<sup>295</sup>
<b>III Non-hematologic neoplasms</b>	
Carcinoma of the gastrointestinal tract	<sup>275, 276, 285, 295</sup>
Carcinoma of the biliary tract	<sup>275, 276, 285, 295</sup>
Carcinoma of the liver	<sup>275, 282, 295</sup>
Carcinoma of the breast	<sup>275, 285, 295</sup>
Carcinoma of the ovary	<sup>276</sup>
Carcinoma of the uterus	<sup>275, 282</sup>
Carcinoma of the prostate	<sup>275, 276, 295</sup>
Carcinoma of the bladder	<sup>275, 282</sup>
Carcinoma of the lung	<sup>275, 282</sup>
Malignant melanoma	<sup>275, 295</sup>
Angiosarcoma	<sup>282</sup>
Oligodendroglioma	<sup>273</sup>
Thymoma	<sup>291</sup>
<b>IV Miscellaneous conditions</b>	
Biliary tract disease	<sup>275, 279, 285, 295, 299</sup>
Acute porphyria	<sup>282</sup>
Gaucher's disease	<sup>286</sup>
Hyperlipemia and xanthomatosis	<sup>288</sup>
Sarcoidosis	<sup>273</sup>
Paget's disease of bone	<sup>275</sup>
Hyperparathyroidism	<sup>271</sup>
Parathyroid adenoma	<sup>267</sup>
Pulmonary fibrosis	<sup>297</sup>
Chronic lung disease	<sup>295, 297</sup>
Polyneuropathy	<sup>297</sup>
Pyoderma gangrenosum	<sup>288</sup>

the association of serum M-components and various types of nonreticular neoplasms is a chance event, especially when one considers that the age adjusted rates for malignant disease in this group of patients may be as high as 20%.<sup>295</sup> Alternatively, both may be secondary to a common stimulus, such as an oncogenic virus.

3. Hereditary factors may be of importance since several family members with the same monoclonal macroglobulin have been described.<sup>262,289</sup>



## Clinical Features

The clinical settings in which BMH is found are evident from Table 53-2. While some individuals appear to be completely healthy, others suffer from associated malignant conditions, chronic infections, "auto-immune" diseases, and chronic pulmonary disease, frequently characterized by fibrosis. Occasionally, transient M-components are seen in association with nonmalignant conditions. By definition, *none* of these patients suffers from malignant disease involving the lymphoreticular system and the monoclonal proteins do not contribute to the clinical features of the patient's illness. Skeletal destruction is not seen.

## Laboratory Findings

The peripheral blood reflects the hematologic complications of the associated disease. The bone marrow generally contains fewer than 10% plasma cells and often the number is well within normal limits. The serologic characteristics of the M-components are identical to those of the malignant plasma cell dyscrasias (Chapter 52), but the normal immunoglobulins are not usually decreased. The incidence of Bence Jones proteinuria is low<sup>270</sup> and when present the amount of protein is rather small. As a consequence, renal impairment is rarely present. When present it is usually due to immune complexes involving the M-component.<sup>278</sup>

## Differential Diagnosis

BMH must be differentiated from other plasma cell dyscrasias and this is usually possible on clinical grounds alone. The concentration of the M-component must be measured from time to time. When a plasma cell dyscrasia cannot be demonstrated, a careful search must be made for an associated malignant lesion.

## Prognosis and Therapy

In patients without associated disease the prognosis is quite favorable except in the few,

perhaps 5%, who eventually develop myeloma. Only after a plasma cell dyscrasia has developed should therapy be instituted. Available evidence is insufficient to determine whether or not treatment benefits those with truly benign monoclonal hypergammaglobulinemia, or delays or eliminates the appearance of malignant plasma cell dyscrasias.

## Cryoglobulins (Cryoglobs)

### Definition

Cryoglobulins are serum protein or protein complexes that undergo reversible precipitation at low temperatures.<sup>343</sup> Such proteins were first described by Wintrobe and Buell in 1933<sup>367</sup> and were termed "cryoglobulins" by Lerner and Watson in 1947.<sup>333</sup> Since several serum proteins, including fibrinogen,<sup>325,327,344,354,358</sup> may become cryoprecipitable under appropriate conditions,<sup>321</sup> it is preferable to use the term "cryoimmunoglobulins" (cryoglobs) when describing antibody molecules with this property.

### Immunochemistry

Immunochemically diverse types of cryoglobs occur in a variety of neoplastic, inflammatory, and infectious diseases (Table 53-3) and are readily subdivided into two main categories<sup>321</sup>:

1. About 25% of all cryoprecipitates consist of *monoclonal immunoglobulins* only, usually derived from patients with plasma cell dyscrasias. In multiple myeloma, cold precipitability is usually associated with monoclonal IgG proteins,<sup>321,341</sup> although a few instances of cold precipitable Bence Jones proteins<sup>301,321,334,362</sup> and IgA proteins<sup>302,321</sup> also have been described. In Waldenström's macroglobulinemia, chronic lymphatic leukemia, and non-Hodgkin's lymphoma, the cryoglobulins usually have the characteristics of IgM.<sup>305,306,312,321,337,341</sup> When monoclonal cryoglobs are found in the absence of other discernible disease, patients are said to suffer

**Table 53-3. Cryoimmunoglobulinemia**

<b>I Monoclonal cryoglobs</b>	
A	Essential or idiopathic <sup>321,341,342</sup>
B	Multiple myeloma <sup>301,302,321,334,341</sup>
C	Waldenström's macroglobulinemia <sup>312,321</sup>
D	Lymphatic leukemia, lymphosarcoma <sup>305,306,341</sup>
<b>II Mixed cryoglobs (monoclonal polyclonal or polyclonal-polyclonal)</b>	
<b>A Autoimmune disorders</b>	
1	Systemic lupus erythematosus <sup>311,321,324,345,359</sup>
2	Arthralgia-purpura nephritis syndrome <sup>321,341</sup>
3	Immune complex nephritis <sup>317,338</sup>
4	Allergic vasculitis <sup>314</sup>
5	Rheumatoid arthritis <sup>329,341</sup>
6	Polyarteritis nodosa <sup>301,329</sup>
7	Sjögren's syndrome <sup>329,341</sup>
8	Thyroiditis <sup>340</sup>
9	Ulcerative colitis <sup>350</sup>
<b>B Chronic infections</b>	
1	Infectious mononucleosis <sup>304,310,365</sup>
2	Cytomegalovirus infection <sup>328,343</sup>
3	Toxoplasmosis <sup>345</sup>
4	Syphilis <sup>321</sup>
5	Lymphogranuloma venereum <sup>321</sup>
6	Leprosy <sup>321</sup>
7	Kala-azar <sup>321</sup>
<b>C Miscellaneous</b>	
	Chronic liver disease <sup>317,343,343</sup>
	Sarcoidosis <sup>341</sup>
	Acute myocardial infarction <sup>318</sup>

from "essential monoclonal cryoglobulinemia." Sera from such patients usually contain IgG cryoglobs.<sup>321,363</sup> The exact relationship of this disorder to multiple myeloma is unclear, although the nature of the protein and the frequent finding of marrow plasmacytosis<sup>316</sup> suggest that this is a plasma cell dyscrasia, perhaps akin to "benign" monoclonal gammopathy (page 1638). Some patients with "essential cryoglobulinemia" eventually develop a malignant plasma cell dyscrasia, usually multiple myeloma.<sup>321</sup>

2. Most cryoglobs (75%) precipitate as immunoglobulin complexes in which one component, usually IgM, but sometimes IgG or IgA, exhibits antibody activity against the second component, which is always an IgG molecule.<sup>321</sup> Such complexes are termed "mixed cryoimmunoglobulins." In about one fifth of these complexes, the anti-immunoglobulin component is a monoclonal protein,

but, in most (four fifths), both components are of polyclonal origin. Occasionally, other proteins such as complement components also form part of the complex.<sup>311</sup> Mixed cryoglobs occur predominantly in association with connective tissue diseases, acute and chronic infections, and a variety of other disorders ("secondary mixed cryoglobs").<sup>321</sup> In such cases, cryoglobs do not possess any antibody activity besides their rheumatoid factor (anti-immunoglobulin) activity,<sup>321</sup> although occasional cases of cryoprecipitable cold agglutinins have been described.<sup>54</sup> Sometimes mixed cryoglobulins containing a monoclonal component are also found in association with plasma cell dyscrasias<sup>321,341</sup> and sometimes they occur in the absence of any discernible underlying disease ("essential mixed cryoglobs").<sup>321</sup>

### Physical and Chemical Characteristics

The physical and chemical characteristics of cryoimmunoglobulins are similar to those of normal immunoglobulins,<sup>321</sup> although the incidence of immunoglobulins and their subclasses and the ratio of  $\kappa$  to  $\lambda$  chains may not follow normal distribution patterns. Thus monoclonal IgM cryoglobs are almost invariably associated with  $\kappa$  light chains and the IgG3 subclass occurs with unusual frequency.<sup>321</sup> Nevertheless, the phenomenon of cryoprecipitation cannot be explained on the basis of unique characteristics of size, shape, or charge. An intact molecule is required for cryoprecipitation since cleavage of interchain disulfide bridges (see Chapter 7) usually results in complete loss of cryoprecipitation activity.<sup>321</sup> It has been suggested that cryoprecipitability is determined by properties resident within the variable region (Chapter 7) of light and/or heavy chains. The mechanisms of cryoprecipitation are unclear but indirect evidence suggests that molecular peculiarities of charge, conformation, and the state of hydration are implicated,<sup>341</sup> and abnormal protein-water interactions appear to be particularly important.<sup>356,357</sup> The interactions are, in general, quite weak and are easily disturbed by mild changes in experi-

mental (in vitro) conditions such as temperature, protein concentration, pH, and ionic strength. Temperature appears to be the most important variable and that at which precipitation starts may range from 35° to 5° C. For any single protein, however, its concentration is also important: in general, the higher the protein concentration, the higher the temperature at which precipitation occurs.<sup>321,341</sup> In addition, the presence of other serum proteins may be a factor in cryoprecipitation, since they apparently help to solubilize cryoIgs.<sup>321</sup> In most cases a pH optimum of 5.5 to 8.0 has been observed and most proteins show decreased solubility with decreasing salt concentrations in the range of 0.05 to 0.3 M.<sup>321</sup> Above 0.3 M, most cryoIgs tend not to precipitate.

## Clinical Features

### Monoclonal CryoIgs

Less than 5% of myeloma proteins and about 7% of Waldenström's macroglobulinemia proteins are cryoprecipitable.<sup>321</sup> Symptoms directly attributable to such proteins are variable and depend at least in part on the temperature and rate at which cryoprecipitation occurs on cooling, a characteristic that is unique for each cryoprotein.<sup>341</sup> The amount of cryoprotein appears to be less important in vivo than in vitro.<sup>341</sup> In one study, two thirds of all patients had no symptoms at all, even in the winter.<sup>341</sup>

Most symptoms result from impaired blood flow due to cryoprecipitation within capillaries.<sup>341</sup> In patients with monoclonal IgM cryoglobulins, the high intrinsic viscosity of these molecules is greatly amplified at lower temperatures and contributes considerably to the poor circulation. Other symptoms of hyperviscosity (page 1626) may also result. The consequences of impaired blood flow are particularly noticeable within the extremities, which may develop cold intolerance, acrocyanosis, tingling, and numbness. Patients may also suffer from livedo reticularis, cutis marmorata, and a typical Raynaud's phenomenon. With time, trophic

changes such as leg ulcers and even gangrene of digits may develop. Dependent purpura is common, but retinal hemorrhages, epistaxis, hemoptysis, and melena may also occur. In addition, thrombosis of major vessels such as pulmonary, renal, and mesenteric arteries has been described.<sup>348</sup> Occasionally, IgM cryoIgs are associated with cold agglutinin activity.<sup>336</sup>

Exposure to cold may also provoke constitutional symptoms such as chills and fever, dyspnea, and diarrhea.<sup>343</sup> Cold urticaria is rarely seen<sup>321</sup> and is associated with complement activation and mast cell degranulation, probably through anaphylatoxin production.<sup>313</sup> Cold urticaria has been transferred passively to normal recipients with whole serum and isolated cryoIgs.<sup>321</sup>

Cryo-Bence Jones proteins usually do not give rise to symptoms and are largely of physicochemical interest.<sup>321</sup>

### Mixed CryoIgs

Two clinical situations, systemic lupus erythematosus and arthralgia-purpura nephritis syndrome, have been most clearly defined.

In *systemic lupus erythematosus*, mixed cryoglobulins are frequently associated with clinically active disease, including renal involvement.<sup>321,321,345,359</sup> These cryoprecipitates most commonly contain IgG, IgM, and the C1q component of complement which is essential for cryoprecipitation, since the latter is abolished by heating for 30 minutes at 56° C. The IgM component is polyclonal and has anti-IgG activity that may be restricted in specificity to the patient's own IgG. Cryoprecipitates containing DNA and IgG anti-DNA antibodies also have been described.<sup>361,319,332</sup> In lupus patients with cryoIgs there appear to be a higher incidence of nephritis and lower complement levels than in those without cryoIgs.<sup>359</sup>

In the *arthralgia-purpura nephritis syndrome*,<sup>321,341</sup> cryoprecipitates containing IgG and IgM, only, have been described. The IgM molecule has rheumatoid factor (anti-IgG) activity and is essential for cryoprecipitation. The IgG can be derived from any

source. One patient with an IgG anti-IgG complex has also been described.<sup>341</sup> Cryoprecipitability is unaffected by heating at 56° C for 30 minutes, although total serum complement activity frequently is low. The clinical manifestations consist of purpura, arthralgia, and weakness in the early stages, and in some patients there is diffuse glomerulonephritis with acute renal failure, frequently with a fatal outcome.<sup>321,341</sup> Both IgG and IgM deposits have been detected in glomeruli in a lumpy-bumpy pattern reminiscent of immune complex disease.<sup>321</sup> There may be hepatosplenomegaly and lymphadenopathy and clinical evidence of autoimmune disorders, such as Sjögren's syndrome and thyroiditis; some patients have antinuclear antibodies.

Mixed cryoIgs also occur in well-defined infections, such as infectious mononucleosis (Chapter 43), in the postperfusion syndrome due to cytomegalovirus infection (Chapter 43), as well as in secondary syphilis, lymphogranuloma venereum, leprosy, kala azar, and other disorders.<sup>321</sup>

## Diagnosis

Proper collection of serum is essential for the detection of cryoIgs, particularly the mixed variety. Blood should be collected in warm syringes and clotted at 37° C. After centrifugation at room temperature or higher, serum is promptly harvested and then stored at 4° C. Monoclonal cryoIgs usually appear within 24 hours or less, but mixed cryoIgs may not appear for several days and a minimal incubation period of 72 hours is essential. When serum is stored in capillary tubes, a cryocrit (volume of cryoIgs/total serum) can be estimated after centrifugation.<sup>321</sup> Finally the isolated cryoglobulin is washed in the cold, redissolved, and subjected to study by immunoelectrophoresis and other immunochemical techniques (see Chapter 7). The cryoprecipitate should be tested for rheumatoid factor activity.

When the concentration of cryoIgs is very high, precipitation or gelling may occur immediately on withdrawal, even when the

syringe is warmed to 37° C. This may be prevented by the use of chelating anticoagulants such as EDTA or ACD.<sup>321</sup> The plasma is then recalcified after its separation from the red cells and the serum is removed at 37° C without significant loss of cryoprecipitate.<sup>321</sup>

Small quantities of cryoglobulins are frequently found in the sera of normal individuals in concentrations of up to 80 µg/ml.<sup>314</sup>

## Therapy

*General measures* such as avoidance of cold cannot be overstressed. Cyproheptadine, an antagonist of histamine and 5-hydroxytryptamine, is useful in the treatment of cold urticaria.<sup>366</sup> In monoclonal cryoimmunoglobulinemias due to *plasma cell dyscrasias*, treatment is primarily directed at the underlying disease. *Plasmapheresis* (page 1629) is of limited benefit, except in monoclonal cryoIgM, in which there is primarily intravascular distribution (Chapter 7). *Corticosteroids* have been effective in a few patients with monoclonal cryoimmunoglobulinemia, presumably because of their anti-inflammatory effects.<sup>322,347,365</sup> Basic organic amines<sup>330</sup> and sulfhydryl compounds<sup>331,353</sup> have been tried but cannot be recommended. The usefulness of cytotoxic drugs such as cyclophosphamide and phenylalanine mustard has not been adequately evaluated, but in isolated instances good results have been achieved, both in essential monoclonal cryoimmunoglobulinemia<sup>307</sup> and in mixed cryoglobulinemia.

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## *Complications of Neoplastic Diseases of the Hematopoietic System and their Treatment*

### **Complications Due to Deficient Number or Abnormal Function of Hematopoietic Cells**

#### **Fever and Infection**

Distinguishing between Fever of Infection and Fever of Neoplastic Diseases without Infection

Evaluation of the Patient with Fever  
Management of Fever without Obvious Infection

Infection in Neoplastic Diseases of the Hematopoietic System

#### **Hemorrhage**

Thrombocytopenia

Disseminated Intravascular Coagulation  
Other Causes of Hemorrhage

#### **Abnormalities of Erythrocytes**

Anemia

### **Complications Due to Infiltration of Organs**

Neurologic Involvement

Ocular Disease

Ears, Nose, Throat, Larynx, and Oral Cavity

The Lungs

Cardiovascular System

Liver

Spleen and Lymph Nodes

Gastrointestinal Tract

Renal Complications

Genitourinary Tract

Endocrine Glands

Musculoskeletal System

Skin

### **Complications Due to Metabolic Imbalance**

Hyperuricemia

Hypercalcemia

Serum Proteins

Pregnancy

**T**HE leukemias, lymphomas, myelomas, and related diseases cause morbidity and mortality through three general mechanisms: (1) a deficit in normal cell number or function (eg, infection due to neutropenia or to deficient production of immunoglobulins); (2) invasion of vital organs with impairment of organ function (eg, Hodgkin's disease invading the lung); and (3) systemic disturbance, as manifested by weight loss, pruritus, or metabolic alteration, such as hypercalcemia. The frequency of these complications differs in the various diseases discussed in Chapters 47 through 53. Nevertheless, quite similar complications may be encountered in all of them and for this reason the complications will be discussed here. Treatment may induce ill effects and these must be distinguished from complications associated with the natural course of these diseases. The therapeutic complications are discussed briefly in this chapter, but their expected frequency with various forms of therapy is considered in Chapter 55.

### **Complications Due to Deficient Number or Abnormal Function of Hematopoietic Cells**

Infection, due to neutropenia or immunologic deficiency, hemorrhage, usually due to thrombocytopenia, and anemia are the chief

causes of morbidity in patients with hematologic malignant diseases.

## Fever and Infection

Infection is one of the major causes of death (Table 54-1) in patients with any of the diseases under consideration, and the infections are quite often fulminant. Consequently, the occurrence of fever in a patient with leukemia, lymphoma, or myeloma always raises the question of infection. However, fever can develop as a manifestation of the neoplastic disease in the absence of infection in a patient with any of the diseases under consideration<sup>29, 30, 37, 102</sup> with the probable exception of chronic lymphocytic leukemia (CLL). Additionally, fever may be due to noninfectious complications such as transfusion reaction, splenic infarction, drug toxicity, and thrombophlebitis. Consequently, prompt, careful, and repeated inquiry into the cause of fever is required if intelligent management of the patient is to be maintained.

### Distinguishing between Fever of Infection (FI) and Fever of Neoplastic Disease (FND) without Infection

The likelihood that fever is caused by the disease (FND) rather than by infection (FI) is greater in one disease than another and differs also according to the stage of the disease in question (Fig. 54-1). Thus, chronic lymphocytic leukemia (CLL) is a disease not associated with FND. We have yet to observe fever in a patient with CLL that could not

be explained by infection or by some other complication.<sup>2</sup> In contrast, infection is an uncommon cause of fever in Hodgkin's disease (HD) until the terminal stages are reached or unless exceedingly vigorous therapy has been employed (Chapter 50). Fever in a patient with HD, sometimes of a characteristic type, is a manifestation of the disease itself (Chapter 50) and its presence at the time of diagnosis has been associated with a poor prognosis.<sup>79</sup>

In chronic myelocytic leukemia (CML), fever is uncommon and, indeed, until the late stages, neither FND nor FI is expected unless severe neutropenia has been induced by therapy (Chapter 48). However, the onset of blastic crisis in CML is commonly accompanied by fever (Chapter 48); on the other hand, in the terminal stages of blastic crisis, neutropenia and infection are also frequent.

In either acute lymphoblastic (ALL) or myeloblastic (AML) leukemia, infection is common<sup>102</sup> and so is FND. The same is true of multiple myeloma (MM) and related diseases, and non-Hodgkin's lymphoma (NHL). Thus, in these diseases no assumptions as to the cause of fever should be entertained when the temperature is found to be elevated.

As disease advances, the frequency of infection as a cause of fever increases (Fig. 54-2). This would be anticipated from the considerations in HD and CML, mentioned above, but it is equally applicable to the acute leukemias.<sup>29, 30</sup>

### Evaluation of the Patient with Fever

*Clues to infection as a cause of fever* cannot be found by inspection of the fever curve.<sup>30</sup> Although there is a tendency toward less hectic fever curves and for the pulse rate to be elevated to a lesser degree in FND than in FI, this has no predictive value in the individual patient.<sup>30</sup> In acute leukemia (AL) there is a rough, positive correlation between the severity of neutropenia and the number of neutrophils entering an induced exudate and the frequency of infection.<sup>28, 64</sup> Thus, in a severely neutropenic patient with fever it would be expected that infection was present.

**Table 54-1. Cause of Death in 450 Patients with Leukemia and Lymphoma—Autopsy Studies at the National Cancer Institute, 1965–1971\***

	Percent
Infection alone	69
Infection and hemorrhage	10
Hemorrhage alone	11
Other causes	10

\*Adapted from Levine<sup>74</sup>

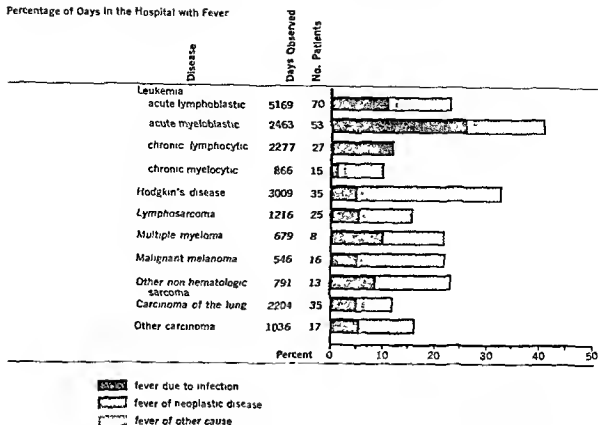


Fig 54-1. The frequency of fever due to infection or due to the malignant disease in patients hospitalized at the National Cancer Institute (From Boggs,<sup>29</sup> courtesy of the author and Medical Science.)

The presence of frank, shaking chills is helpful as they are much more common with FI than with FND.<sup>6,30</sup> A careful physical examination is most important, including a rectal examination; *rectal infection* is fairly common in neutropenic patients and often is unsuspected by either patient or physician until an area of tenderness is detected on digital examination.<sup>115</sup> Abdominal tenderness is always alarming since serious infection, often secondary to ulceration of the bowel, may be heralded by this finding.<sup>31</sup> In all instances of unexplained fever, an x ray of the chest should be obtained, even if physical examination or the history fails to indicate pneumonia, because an early infiltrate may be relatively asymptomatic. If a new infiltrate is detected, it is best to assume that it indicates infection (page 1657).

Laboratory examinations that should be made routinely in all febrile patients include culture of blood and urine for bacteria, fungi, and viruses. Septicemia, in the absence of a demonstrable portal of entry (*lanthanic septi-*

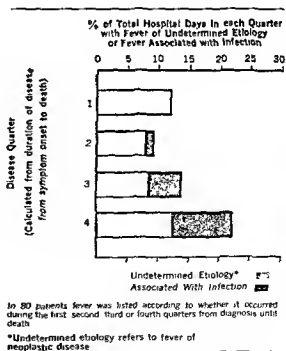


Fig 54-2. The frequency of fever during various stages of disease in hospitalized patients with leukemia, lymphoma, or myeloma (From Boggs,<sup>29</sup> courtesy of the author and Medical Science.)

emia), is not unexpected<sup>30,32</sup> and, in neutropenic patients, urinary tract infection may occur without the expected increase in leukocytes in the urinary sediment.<sup>30</sup> Unless there are symptoms suggesting disease of the respiratory or gastrointestinal tracts, routine cultures of pharyngeal secretion, sputum, or stool usually are not helpful.

Unless a specific infection is suggested by the examination or another cause of fever is uncovered by the above-mentioned studies it usually turns out that FND is present. One exception to this suggestion should be stressed. In the patient with lanthanic septicemia the only clue may be the sudden development of general signs of toxicity. The patient who suddenly appears to be quite ill is likely to have septicemia. The development of lanthanic septicemia can be detected with some accuracy if the diagnosis is kept in mind.<sup>29,32</sup> Of 55 patients with fever without an evident focus of infection who were evaluated before the results of blood cultures were known, six were thought to have lanthanic septicemia. Blood cultures proved to be positive in four of these, while positive blood cultures were found in only one of 49 not thought to have septicemia.<sup>32</sup>

The cause of FND is unknown. The presence of fever correlates with the presence of active disease, but it does not reflect the severity of disease with any accuracy<sup>30</sup> (Fig. 54-2). An unidentified substance that produces fever when injected into rabbits was found in the urine of febrile patients with HD, but was not present in normal urine.<sup>124</sup>

#### Management of Fever without Obvious Infection

Antibiotics should not be used simply because fever is present. A controlled clinical trial of tetracycline versus a placebo<sup>32</sup> in FND and a study of the use of ampicillin<sup>45</sup> suggested that routine antibiotic therapy may be harmful because of its role in changing bacterial flora. Thus, if no evidence of infection is detected after careful evaluation and lanthanic septicemia is not suspected, antibiotics should be withheld. However, reevalua-

tion of the febrile patient should be carried out each day. The symptoms induced by FND are quite variable; some patients remain relatively asymptomatic despite the presence of high fever, while others suffer severe prostration. Treating patients with fever by giving aspirin may provide symptomatic relief for some, but in others the sudden temperature drop induced by aspirin with its frequent attendant diaphoresis actually leads to increased morbidity, especially weakness. Indomethacin therapy may lead to fairly long-term control of fever, particularly in patients with HD.<sup>121</sup> Steroid therapy will usually control fever of any cause, at least for a few days,<sup>30</sup> but because of potential side effects this should not be used for fever *per se*.

#### Infection in Neoplastic Diseases of the Hematopoietic System

In managing patients with hematologic neoplasms the accurate diagnosis and treatment of infection can be as important as antitumor therapy (Chapter 55).

#### Defects in Host Resistance Leading to Increased Frequency of Infection

The natural course of the diseases under consideration eventually includes a phase of impaired host defense (Table 54-2). This includes impairment of phagocytic defenses (usually neutropenia), and defective circulating antibody production (humoral immunity) and impaired cellular immunity, or combinations thereof. Antitumor therapy induces similar impairment of the host's defensive mechanisms.

Defects in other aspects of the antimicrobial defense system have been described, but their frequency and overall contribution to the problem of decreased host defense are poorly defined. A reduced rate of phagocytosis of aggregated albumin, carbon, or other particles by the reticuloendothelial system has been reported in some patients with AL, CLL, or MM, but was less common in those with CML or any of the lymphomas.<sup>17,61,92</sup> In one study,<sup>116</sup> antibacterial

**Table 54-2. Usual Causes of Reduced Host Defense during the Natural Course of Neoplastic Diseases of the Hematopoietic System**

Disease	Neutropenia	Defect in Host Resistance	
		Reduced Circulating Antibodies	Impaired Cell Mediated Immunity
Acute myeloblastic leukemia	Common	Rare	Rare
Acute lymphoblastic leukemia	Common	Rare	Rare
Chronic lymphocytic leukemia	Uncommon	Common	Common
Chronic myelocytic leukemia	Rare	Rare	Rare
Blastic crisis of CML	Common	Rare	Rare
Multiple myeloma	Uncommon	Common	Rare
Hodgkin's disease	Rare	Rare	Common
Lymphocytic lymphoma	Uncommon	Common	Common
Histiocytic lymphoma	Rare	Uncommon	Uncommon

activity of native serum was normal for gram-positive bacteria in patients with CLL, CML, or HD, but was abnormally low for gram-negative bacteria. In other studies, normal or increased antibacterial activity of sera was reported in association with all the diseases, but there was disagreement as to which type of disease is characterized by increased activity.<sup>77,112</sup> In monocytic leukemia,<sup>100a</sup> increased bactericidal activity appears to be due to hyperlysozymemia. The role of properdin in antibacterial defenses remains to be clarified<sup>43</sup> and there is disagreement as to whether low<sup>44</sup> or normal<sup>41</sup> levels are found in patients with leukemia, lymphoma, or myeloma. To some extent this discrepancy may reflect differences in methodology since assays involving zymosan or bacteriophage gave different results in the same patients.<sup>14</sup> Activation of the complement system (C1 through C7) is necessary for the full development of inflammation and is crucial to chemotaxis and phagocytosis<sup>106</sup> (Chapter 6). However, since most hereditary abnormalities of the system are not associated with increased susceptibility to infection,<sup>106</sup> measurement of variation in complement levels in some patients with leukemia is of questionable significance.

The expected defect in host defense in each disease is summarized in Table 54-2 and is considered in more detail below.

*Neutropenia* (less than  $1.8 \times 10^9/l$  segmented and band-form neutrophils) is a common complication of AL and low normal neutrophil levels are characteristic of multiple myeloma, but neutropenia is not frequent during untreated phases of other hematologic neoplastic diseases. When neutropenia occurs in patients with HD or NHL, in the absence of suppressive therapy, marrow invasion with tumor or fibrosis of marrow is usually present and anemia and thrombocytopenia are likely to be found in addition to neutropenia. In patients with CML, neutropenia occurs only with blastic crisis, in the face of developing myelofibrosis, or with therapy. Although it has been stated that neutropenia is common in patients with CLL,<sup>44</sup> careful differential counts reveal it to be unusual in untreated patients.<sup>2,120</sup> If, in making the differential count, 500 or 1000 leukocytes are counted, most untreated patients with CLL are found to have normal absolute numbers of neutrophils.

There is some correlation between the degree of neutropenia and the frequency of infection, although it is difficult to define a critical neutrophil level.<sup>2,27,28,30,64</sup> In any of the diseases under consideration, neutropenia is almost always the result of decreased production. When neutropenia is severe, exudates may be devoid of neutrophils,<sup>2,64,66</sup> presumably hindering localization of infec-

tion and explaining the increased frequency of septicemia in such patients.

*Functional defects in neutrophils* (Chapter 42) have also been described in certain circumstances. Some disagreement exists as to whether or not mature neutrophils ingest organisms at a normal rate in patients with CML, but if any defect exists it is minor.<sup>30,121</sup> Phagocytosis is normal in other diseases,<sup>73,122,123</sup> but some defects in killing ingested organisms have been described. Patients with advanced HD and acute leukemia have been reported to have defective candidacidal activity<sup>71</sup> and other bacterial-killing defects have been reported in patients with acute leukemia, especially during chemotherapy.<sup>124</sup> Complete absence of myeloperoxidase was reported in neutrophils from two patients with AML.<sup>47,101</sup> Functionally deficient neutrophils may be found in a number of patients with AML or blastic crisis of CML<sup>42,42a</sup> and defective bactericidal activity by mononuclear phagocytes is common in acute myelomonocytic leukemia, as well as in some patients with lymphoma.<sup>42b</sup> One study suggested that a serum defect might contribute to defective accumulation of neutrophils in exudates in AML subjects.<sup>64</sup> In patients with CML, the total number of neutrophils migrating into induced exudates is normal or even increased.<sup>15</sup>

*Therapy of patients with neutropenia* depends upon the cause of this condition. There is no effective way of stimulating increased neutrophil production. If neutropenia is thought to be due to chemotherapy (Chapter 55), use of the toxic drugs should be discontinued until neutropenia disappears. Conversely, if the neutropenia is thought to be due to the malignant disease, the use of the same drugs may produce a remission of the underlying disease and correct the neutropenia. Leukocyte transfusion is discussed in Chapter 12, and on page 1661.

*Deficient immunoglobulin production* (Chapter 44) is the second major cause of frequent and severe infection in patients with certain neoplastic diseases. Impaired immunoglobulin synthesis in those with CLL frequently is a major cause of infection.<sup>72,76,87,120</sup> A

similar deficit is demonstrable in the majority of patients with MM<sup>53a,76,78</sup> and in a smaller percentage of those with lymphocytic lymphoma.<sup>76,87</sup> Except in severely ill patients, it is unusual in those with other lymphomas<sup>21,87</sup> or HD.<sup>11,76,87</sup> Antibody formation appears to be normal in most patients with acute leukemia<sup>20,51,72,76,104</sup> or with CML<sup>72,76</sup> unless extensive therapy has been employed.<sup>51,62,84</sup>

Defective production of circulating immunoglobulin is often reflected in hypogammaglobulinemia (less than 0.8 g/dl of serum) and by reduction in naturally occurring immunoglobulins such as isoantibodies to red cell antigens.<sup>54,120</sup> If the serum gamma globulin is reduced, defective antibody formation is expected, but the converse is not necessarily true; i.e., defective antibody production may be present in patients with normal levels of gamma globulin.<sup>120</sup> Increased catabolism may also contribute to hypogammaglobulinemia in MM.<sup>121a</sup>

Hypogammaglobulinemia correlates to some degree with duration and severity of disease.<sup>11,120,131</sup> (Fig. 54-3). However, hypogammaglobulinemia may be observed in the absence of extensive CLL or MM and such patients may die of infection at a time when their disease seems to be in a fairly early stage. All classes of immunoglobulins may be depressed or there may be selective depression of one class.<sup>94,104</sup> Normal levels of salivary IgA have been reported in CLL and MM patients in whom serum levels were depressed.<sup>94</sup> Quantitative immunoglobulin levels are normal in most patients with CML,<sup>56</sup> as they are in untreated patients with acute leukemia,<sup>62a</sup> but all classes may be depressed by therapy.<sup>101</sup>

Therapy of defective hypogammaglobulinemia *per se* is quite unsatisfactory. The use of pooled gamma globulin in treating patients with infection is discussed on page 1662. Therapy of MM may lead to improved gamma-globulin levels in a few patients and this has also been noted in HD patients (see Fig. 50-12). However, no improvement in humoral immunity in CLL patients has been reported with any form of therapy.

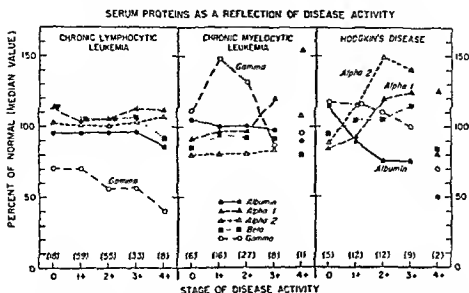


Fig 54-3. Relationship of the severity of CLL, CML, and HD to serum protein levels. Note that in CLL while levels of gamma globulin decline with increasing severity, other serum proteins do not. Numbers in parentheses are the numbers of observations at each stage of disease. (From Boggs and Fahey,<sup>30a</sup> courtesy of the authors and Journal of the National Cancer Institute.)

*Cell-mediated immunity* is most commonly measured by the patient's ability to react with delayed hypersensitivity to a subcutaneously injected antigen or by lymphocyte conversion *in vitro* in the presence of phytohemagglutinin (PHA). As discussed in Chapter 7, these measures usually correlate with one another and with still other measures. This is usually true in HD; i.e., the patient who is anergic as judged by loss of skin sensitivity to agents such as old tuberculin has lymphocytes that fail to convert when exposed to PHA or to pokeweed mitogen.<sup>42c</sup> However, such correlation is not always present; a normal response to the mixed lymphocyte reaction has been seen in patients whose PHA response was abnormal.<sup>71</sup> The patient with CLL usually shows reduced or absent lymphocyte conversion with PHA,<sup>134</sup> but he does not lose skin hypersensitivity to antigens such as old tuberculin.<sup>120</sup> In spite of this, CLL patients often fail to respond to new antigens such as dinitrochlorobenzene.<sup>83</sup> Patients with HD fail to respond to new antigens and lose prior sensitivity as well.<sup>83</sup>

Thus, in both CLL and HD a defect in cellular immunity is often present, but there

are some differences in the nature of the defect. It appears that patients with HD fail to mount either a primary or a secondary immune response while those with CLL maintain secondary responses, but cannot mount a primary response. Cell-mediated immune responses may be abnormal in NHL or MM patients,<sup>70</sup> but these responses have not been as well characterized as in CLL or HD. Anergy is uncommon in acute leukemia.<sup>51,67</sup>

Various studies differ as to the frequency of impaired cell-mediated immunity. This is due at least in part to differences in the stages of disease studied and the sensitivity of the tests used. In HD there is a direct correlation between the frequency of impaired cell-mediated immunity and the severity and stage of disease (Chapter 50).

Although therapy with a wide variety of antitumor drugs produces anergy as a transient toxic manifestation<sup>12</sup> in either HD or CLL, treatment, if successful, may lead to eventual improvement of cell-mediated immunity.<sup>83</sup> In HD, after apparent eradication of disease by radiotherapy or chemotherapy, cell-mediated immunity as judged by either



skin tests or PHA conversion may return to normal although many months are required in most cases.<sup>83</sup> In CLL, as the blood lymphocyte concentration is reduced by therapy, PHA conversion improves and may revert to normal.<sup>83</sup> Whether this indicates overall improvement in cell-mediated immunity or merely represents the unmasking of a residual population of normally responsive blood lymphocytes remains to be determined.

Splenectomy is now carried out as part of the staging procedure in many patients with HD (Chapter 50). If the hazard of infection is increased by splenectomy, as some hold (see Chapter 45), it does not seem to be increased to a greater degree in HD than in nonmalignant conditions.<sup>49</sup>

### Characteristics of Complicating Infections

The site of infection does not differ appreciably among the various forms of leukemia, lymphoma, and myeloma. Noteworthy in all of these diseases is the frequency with which septicemia may complicate a seemingly trivial infection.<sup>30,113</sup> As judged by one series, the lung is the most common site of serious infection, followed closely by the oral cavity (especially the pharynx), skin,<sup>30</sup> and rectum.<sup>115</sup>

The organisms responsible for infection are quite varied and depend in large part on three factors: recent antibiotic usage, whether the infection was acquired at home or in the hospital, and the nature of the underlying defect in host defense.

It is useful to subdivide infections into three types: *primary infections*, the first infection with a particular organism at a particular site in the absence of antibiotic therapy; *recurrent infections*, developing at the same site or with the same organism after therapy for the primary infection has been discontinued; and *superinfections*, infection that begins while antibiotic therapy is already in use.<sup>30</sup>

Deficiency of humoral immunity most often leads to primary infections with pneumococci, staphylococci, meningococci, *H. influenza*, and sometimes streptococci and

salmonella.<sup>83</sup> Since antibodies are not produced as a result of these infections, recurrent infection with the same organism is common. Unusual causes of infection such as *Pneumocystis carinii* or *Giardia lamblia* may also be primary infecting organisms.<sup>83</sup>

With defective cellular immunity, organisms thought of as "intracellular" pathogens are the common causes of primary infections, namely, the tubercle bacillus, various fungi, and also a variety of viruses as well as salmonella.<sup>83</sup> When combined cellular and humoral defects are present, either intra- or extracellular organisms may predominate.

Organisms associated with primary infection due to neutropenia are similar to those associated with deficiency of humoral antibody except that the gram-negative bacteria, especially *E. coli*, are the organisms that most often are responsible for primary infection.<sup>30</sup> This may reflect infection by flora of the bowel. During hospitalization, patients often become infected with organisms which become part of their flora.<sup>113a</sup> In patients who have recently received antibiotics for FND, primary, recurrent, and superinfections, especially those acquired in the hospital, are more often due to pseudomonas, klebsiella, and proteus.<sup>24,30,38,50</sup> A vicious cycle is established in some patients; the first infection is easily treated; the second is due to a more resistant organism, but is still controlled by vigorous antibiotic therapy; and subsequent infections are caused by organisms against which antibiotics are only partially successful, such as pseudomonas, candida, or aspergillus. This is due, at least in part, to the source of the offending organism. Many and perhaps most patients with leukemia, lymphoma, and myeloma become infected by organisms present in their own normal flora. As the flora, particularly that of the gut, is changed by antibiosis, resistant bacteria and fungi become the predominant species. For practical purposes, the only way in which this cycle can be broken is to induce improvement in host defense, by inducing remission in acute leukemia<sup>102</sup> or by allowing recovery from drug-induced neutropenia. Unfortunately, when reduced circulating immuno-

globulins complicate CLL, NHL, or MM, therapy of the underlying disease may not improve the ability to produce antibodies (page 1654).

Infections with the common bacteria manifest the usual features associated with such infections, with certain exceptions. As previously discussed, septicemia is exceptionally common. Necrotic, ulcerative, and hemorrhagic skin lesions are often noted in pseudomonas septicemia<sup>50</sup> and may be observed occasionally with septicemia due to other organisms in neutropenic patients. In the presence of neutropenia, pus may not appear and blood neutrophil response is absent.

Bacteria that are usually nonpathogenic in the normal population often become pathogens in the compromised host. Thus, *Staphylococcus albus* and nonpigmented *Serratia* are reported causes of infection in a significant number of patients.<sup>24</sup> Clostridial septicemia, an infection usually associated with poorly cared for wounds, may occur de novo in leukemia.<sup>31</sup> The only report of septicemia due to *Corynebacterium equi* was in a patient with NHL.<sup>82a</sup>

*Tuberculous infections*, while uncommon, present a unique problem in diagnosis since the signs and symptoms of tuberculosis are quite similar to those found in the lymphomas; even leukemoid reactions that were due to tuberculosis have been reported (Chapter 41). Reactivation of quiescent tuberculosis may follow loss of cellular immunity due either to disease, especially HD,<sup>83</sup> or therapy. Atypical mycobacteria may also be found to be the cause of infection.<sup>85</sup> At one time, tuberculosis was so frequent in patients with HD that there was strong suspicion that it was the cause of the disease,<sup>4</sup> but, as the frequency of tuberculosis has decreased in the general population, it has become a less common finding in persons with HD.<sup>79</sup>

*Infections with the herpes viruses* are not unusual in patients with depressed cellular immunity and in those receiving steroid therapy. The varicella-zoster virus is particularly troublesome. In 1913, Fieschl reported generalized herpes zoster in a patient with CLL<sup>87</sup> and, in 1924, Pancoast and Pendergrass<sup>93</sup>

called attention to the high incidence of herpes zoster in HD patients. Surveys of large series of patients indicate that infections with the varicella-zoster virus are most common in HD (10% of patients) and occur with increased frequency in NHL and CLL, but in patients with other forms of leukemia or with myeloma they may not greatly exceed the frequency in the general population.<sup>23,119,123,135</sup> Attempts to correlate infiltration of the nerve roots with the presence of localized herpes zoster<sup>23</sup> have been unsuccessful<sup>129</sup>; defective cellular immunity would appear to be the primary fault, particularly when the lesions are disseminated.<sup>68,123</sup> Patients with a prior history of varicella, whether adults or children,<sup>68</sup> may develop local zoster, local zoster followed by varicelliform generalization, or may have a generalized varicelliform eruption initially.<sup>119,123,135</sup> Lesions that remain localized are often very painful, at times becoming superficially gangrenous, and may persist for inordinate lengths of time, but it is the generalized form of the disease that is most serious. Generalized lesions were seen in 14 of 48 instances of herpes varicella infection in adults with HD in one series.<sup>123</sup> Although in that group no deaths were directly attributable to the infection, deaths do occur with such infections.<sup>48,95</sup> Herpes zoster pneumonia, a very rare event, has accompanied generalized herpes in HD.<sup>95</sup>

*Varicella* (chickenpox), the initial manifestation of infection with the varicella-zoster virus, is primarily a disease of children and knowledge concerning this infection in association with hematologic malignant disease is thus limited to ALL. In some patients the course of the disease has not been more severe than in the normal population, but others have developed varicella pneumonia, which occasionally has proved fatal.<sup>25,96</sup> Some of the patients developing this complication were receiving steroids—therapy known to be associated with varicella pneumonia in nonleukemic populations<sup>96,103</sup>—and almost all had been receiving some form of antileukemic therapy.<sup>25,96</sup> Therefore, it is uncertain whether varicella infection when

severe is due to the existence of the underlying disease, to therapy, or to both.

*Herpes simplex* infections are common, particularly in febrile patients, but whether they are more common in leukemia and lymphoma than in other diseases of which fever is a manifestation is uncertain.<sup>91</sup> Severe, spreading simplex infections are observed occasionally and may be varicelliform in character and grossly indistinguishable from zoster (Fig. 54-4). In our experience, severe spreading herpes simplex infections have occurred only in patients receiving steroid therapy.

Severe complications may follow *vaccination for smallpox*, particularly in patients with CLL. Generalized vaccinia,<sup>92 130</sup> or a severe spreading local reaction, vaccinia gangrenosum, may occur<sup>55</sup> and often is fatal. Poor circulating antibody response may be the

primary cause of this reaction since these complications also have been reported in patients with hypogammaglobulinemia due to other causes.<sup>46,55,83</sup> Patients with other forms of leukemia who developed these lesions had been receiving antileukemia therapy.<sup>46</sup> The presence of any form of hematologic neoplasm should be considered a contraindication to smallpox vaccination.

Symptomatic *cytomegalovirus infection* is fairly common in patients with acute leukemia subjected to vigorous therapy, but is much less common in those treated in a less intense fashion<sup>53,105,120</sup>; it is a problem in anergic patients with HD.<sup>81</sup> Localized self-limited infections with this virus occur in most persons. Consequently, isolation of the virus from a single source, such as the urine,<sup>13,106</sup> or serologic evidence of infec-

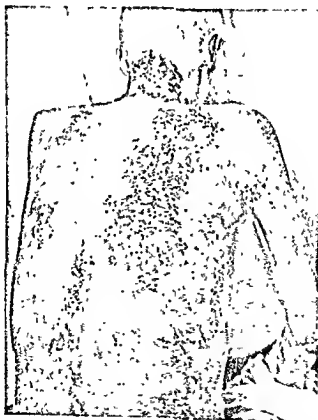


Fig 54-4 Disseminated herpes simplex lesions in a patient with HO (From Muller et al<sup>91</sup> courtesy of the authors and American Journal of Medicine)

tion<sup>126</sup> does not necessarily indicate serious infection. Generalized infection, associated with evidence of pneumonia, may prove fatal and in such patients the virus can be found in many sites such as sputum, blood, urine, and CSF.<sup>105</sup>

Other viral infections of serious nature rarely occur although occasional reports of such severe complications as fatal pneumonia due to natural measles infection or to measles vaccine have appeared.<sup>89</sup> Reports of such infections as lymphogranuloma venereum in patients with CLL<sup>80</sup> are of interest, but an increased frequency of such infection has not been established.

In most instances of infection with opportunistic fungi these organisms are found as pathogens in the setting of depressed host

defense, advanced disease, chemotherapy, and heavy antibiotic usage. For instance, oral moniliasis (Fig. 54-5) occurring in terminally ill patients was shown to be most common in patients receiving antibiotics, antitumor therapy, and steroids; next most common in patients in whom two such factors were present; and least common when only one factor was present.<sup>33</sup> *Torulosis glabrata* has been reported as a pathogen in only 11 patients, but all of these had severe, underlying illness and all but one were receiving antibiotics, steroids, immunosuppressive chemotherapy, or combinations thereof.<sup>82</sup> Evidence has been presented that antibiotics enhance the incidence of candida by altering the fungus and perhaps by changing host resistance as well as altering normal flora.<sup>114</sup>

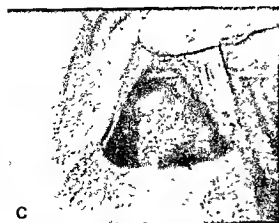
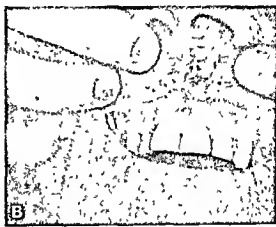
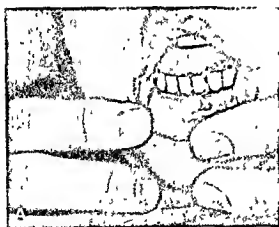


Fig 54-5. Oral moniliasis of lips (A), gingiva (B), hard palate (C), and "bite line" (D) in patients with acute leukemia

*Candida species* probably are the most common cause of serious fungal infections, followed closely if not exceeded by *aspergillus*,<sup>19,86,89</sup> but a variety of other fungi also have been reported.<sup>101,111,136</sup> Proof of infection with these organisms is often quite difficult to obtain because cultural evidence of the presence of fungi in the pharynx, sputum, or stool does not constitute evidence of infection, these organisms often being part of the normal flora.<sup>18,33,101</sup> Furthermore, abscesses attributable to these fungi are often quite difficult to detect.<sup>97,101,136</sup> In one series of 49 patients with acute leukemia, eight had systemic fungal infections at autopsy; in only one of these was the infection detected during life.<sup>48</sup> In another series, 18 of 65 patients had fungal infection at autopsy, but in none of these had the infection been diagnosed during life.<sup>83</sup> Demonstration of budding forms or of pseudomycelia on gram stain constitutes good evidence of significant infection, as does tissue invasion noted in biopsy specimens.<sup>33</sup> The presence in serum of precipitating antibodies against the cytoplasmic "S" antigen of *C. albicans* suggests visceral infection with that organism. However, demonstration of agglutinating antibodies and positive reactions to skin tests are of little diagnostic value unless rising antibody titers are observed since there is a high frequency of positive reactions in the general population.<sup>97,100</sup> Serum levels of antibody against aspergillosis have not proved useful in detecting invasive disease.<sup>139</sup> The detection of cryptococcal antigen in CSF may be useful in diagnosis of infection due to this agent.<sup>59</sup>

By routinely culturing bone marrow aspirates for fungi from patients with leukemia, six otherwise unsuspected systemic infections were uncovered in 187 patients, namely, histoplasma, cryptococcus, and candida.<sup>65</sup>

Most reports of such unusual infections as torulosis have been recorded in patients with HD or CLL.<sup>39,52</sup>

Other types of parasitic infection that are common but usually are asymptomatic in the general population may be serious in patients with depressed host defense, eg, toxoplasmosis.<sup>16,40,81,109,173</sup> *Pneumocystis carinii* infec-

tion also may occur and may be difficult to recognize<sup>107,110</sup> unless techniques such as endobronchial brushing are employed.<sup>104a</sup>

Simultaneous infection with more than one organism is common.<sup>24,30</sup>

### Treatment of Infections

The most critical steps in therapy are isolation of the specific organism, determining its sensitivity to antibiotics, and attempting to improve the underlying defect in host defense. Antibiotic therapy should be as specific as possible, not only to properly combat the infecting organism, but also to change residual host flora as little as possible. Thus, in pneumococcal infection, modest doses of penicillin are preferable to the use of broad-spectrum antibiotics. Since superinfection is common<sup>39</sup> the blood and material from infected sites must be recultured repeatedly in patients who are not responding to therapy in the expected manner. Broad-spectrum antibiotic coverage is necessary in certain circumstances, as in the patient with suspected septicemia in whom results of blood cultures are not yet available. In such a patient, one of the penicillinase-resistant penicillins such as cephalosporin or ampicillin should be coupled with one of the agents possessing broad activity against gram-negative organisms such as gentamycin, colistin, or kanamycin. If the setting is such that pseudomonas infection is likely, large doses of carbenicillin should be added as well.

Patients having systemic fungal infection are usually treated with amphotericin,<sup>19</sup> although those with some strains of candida or cryptococcus may be treated with 5-fluorocytosine,<sup>128</sup> an apparently less toxic drug than amphotericin. Nystatin, applied locally, is of benefit in treating patients with superficial infections such as thrush,<sup>33</sup> but is of no benefit in those with systemic infections since it is not absorbed.

Treatment of patients with viral infections generally is unsatisfactory. Patients with the herpes viruses and complications of smallpox vaccinations may be treated by administering large doses of hyperimmune gamma globu-

lin.<sup>55</sup> Thiosemicarbazones may be useful in disseminated vaccinia.<sup>35</sup> The administration of transfer factor (Chapter 44) holds some promise of correcting failure of antibody formation and has been used with apparent benefit in disseminated vaccinia.<sup>83</sup> No treatment of proven benefit has been reported for cytomegalovirus infection.

Pentamidine is useful in treating patients with severe infections with *Pneumocystis carinii*<sup>732</sup> and pyrimethamine and sulfonamides have been reported to be useful in that infection,<sup>110</sup> as they have in toxoplasmosis.<sup>16</sup>

*Gamma globulin therapy* for acute infections may be tried in patients with deficient circulating immunoglobulins. It is of no benefit in infected, neutropenic acute leukemics<sup>20</sup> since immunoglobulin defenses are usually intact in this disease (page 1654). Whether gamma globulin has any influence on infection in patients with CLL or MM remains to be determined.

*Neutrophil transfusion*, properly performed, is probably of benefit to the infected, neutropenic patient.<sup>24,34,58,74</sup> However, as reviewed in Chapter 12, this experimental procedure is fraught with difficulties. Simply collecting enough neutrophils to give a meaningful dose is difficult and the life span of this cell in normal subjects is so short that daily transfusions would be necessary to more than transiently raise the level of neutrophils.<sup>34</sup> Neutrophils are quite susceptible to in vitro damage so that the majority of infused cells fail to circulate in the recipient unless they have been collected appropriately. Incompatibility of HLA haplotype and naturally occurring or acquired leukoagglutinins are other factors that may make such transfusions ineffective.<sup>58,69</sup> Adverse reactions to leukocyte transfusion may occur, as they do with transfusion of any other blood product (Chapter 12). Transfused lymphocytes have survived for sufficient periods to produce graft-versus-host disease in immunosuppressed patients.<sup>60</sup> When CML leukocytes have been infused, transient engraftment of Ph<sup>1</sup> chromosome-containing cells has been noted in the marrow of patients with AL, but no permanent "takes" of CML have been ob-

served.<sup>60</sup> Toxoplasmosis has been transmitted by neutrophil transfusion.<sup>69,117</sup> For the present, neutrophil transfusion must remain an interesting, but experimental, form of therapy.

### *Prevention of Infection*

A variety of means have been sought to reduce the frequency of infections during periods of defective host resistance.

*Antibiotics given prophylactically* are of proved benefit in conditions in which one is trying to prevent infection with a specific organism, such as prophylaxis for streptococci in patients who have suffered from acute rheumatic fever or for shigella during epidemics of dysentery. However, prophylactic antibiotics are of no proven benefit in avoiding infections from a wide variety of organisms. Indeed, by changing the flora of the host they may well be harmful.<sup>32</sup> The ideal goal of prophylaxis, complete sterilization of the patient, is virtually impossible to achieve unless a "sterile environment" is also provided. However, gut sterilization can be attained in some patients treated in normal hospital environments with a combination of oral, nonabsorbable antibiotics.<sup>99</sup>

"Sterile" environments consist of units such as "life-island" or laminar flow rooms.<sup>24</sup> The laminar flow room is one in which air, sterilized by filtration, flows uniformly from one end of the room, usually from the head of the bed, to the other. The patient's bed, nightstand, etc. are sterilized, but the end of the room toward which air flows is nonsterile. An attempt is made to render the patient germ-free by intense scrubbing of the skin supplemented by the topical application of antibiotics and large doses of oral, non-absorbable antibiotics, and by feeding him sterilized food and water. In some instances, no organisms have been cultured from patients maintained in this manner and the frequency of infection has been reduced as compared to that in patients maintained in a normal hospital environment.<sup>24,135a</sup>

The psychological difficulties associated with this type of isolation may be considerable and the cost of maintaining patients in such an

environment is high. Thus, until it is shown that the maneuver leads to overall benefit, such as a much greater frequency and duration of remissions in AML patients, it should be considered a form of experimental therapy. In this regard, in a controlled trial the frequency and severity of infection were reduced in adults with newly diagnosed acute leukemia who were maintained in a protected environment ("life-island" or laminar flow room) and sterilized with antibiotics to the extent possible, as compared with (1) those having sterilization of the gut without utilizing a protected environment, or (2) those having neither prophylactic antibiotics nor a protected environment.<sup>75</sup> No difference was observed between the patients of the two latter groups. However, neither the frequency nor the duration of remission differed between the groups. "Sterile" environment units may be helpful in ensuring survival during experimental therapeutic procedures such as the administration of lethal doses of irradiation with the object of salvage by bone marrow transplantation (Chapter 55).

*Prophylactic administration of gamma globulin* to patients with reduced serum immunoglobulins due to CLL, NHL, or MM has not been studied in a consistent enough fashion to evaluate.<sup>41</sup> Very large, frequent doses would be required to maintain normal levels in such patients. This would be attended by a significant frequency of allergic reactions as well as hepatitis. However, in such patients who are suffering from severe, recurrent infections a trial of prophylactic gamma globulin therapy would appear to be justified.<sup>47</sup>

*Antituberculous prophylaxis* with isoniazid should be used in any patient with a history of tuberculosis since reactivation may occur due to the underlying disease or to therapy. Such therapy also is advisable if the reaction to the tuberculin skin test is positive, particularly if steroid therapy is used.

## Homorrhage

Serious, life-threatening hemorrhage is a common problem in the acute leukemias and may occur in association with any of the

diseases under consideration. It is usually the result of thrombocytopenia, but may be secondary to platelet dysfunction, disseminated intravascular coagulation (DIC), liver disease, the hyperviscosity syndrome, or still other causes.

## Thrombocytopenia

Reduced numbers of platelets are found in more than 90% of patients with acute leukemia at the time of diagnosis, and modest reductions are observed in a significant number of patients with CLL and MM.<sup>1,2,9</sup> Thrombocytopenia is unusual in untreated patients with lymphoma, unless marrow invasion or marrow fibrosis is present, and when noted in patients with CML it usually indicates impending or overt blastic crisis. However, thrombocytopenia is a common side effect of chemo- and radiotherapy in any of these diseases.

Petechiae and ecchymoses are the most common manifestations and may occur with only modest degrees of thrombocytopenia (Fig. 54-6) (Chapter 34). Life-threatening hemorrhage from thrombocytopenia is usually associated with very severe platelet reduction. Such hemorrhage usually is into the gut or the subarachnoid space, but pulmonary hemorrhage, uterine hemorrhage, and hemorrhage from unusual sites such as the ovary<sup>159</sup> also may cause death.<sup>1</sup> Epistaxis, while troublesome, usually can be controlled by local measures and, while hematuria is fairly common, it is rarely life-threatening.

The relationship of hemorrhage to the degree of thrombocytopenia has been defined in acute leukemia<sup>151</sup> (Fig. 54-6). Ecchymoses and petechiae were found occasionally when platelet counts were above  $50 \times 10^9/l$  and became increasingly more frequent as more severe degrees of thrombocytopenia were encountered. Life-threatening hemorrhage was not observed when platelet counts were above  $50 \times 10^9/l$  and indeed was in no sense common until less than  $5 \times 10^9$  platelets/l were present. Thus, if serious hemorrhage occurs with platelet counts exceeding 20 to  $30 \times 10^9/l$ , other causes contributing to hemorrhage should be searched for.<sup>150</sup>

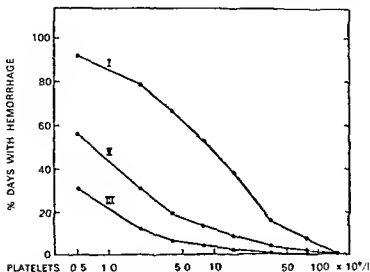


Fig 54-6 Relation between hemorrhage and platelet count. The percentage of days when hemorrhage occurred in the 92 patients combined is shown for each of the levels of platelet count. Curve I shows data for all hemorrhagic manifestations. In curve II skin hemorrhage and epistaxis have been excluded. Curve III refers only to grossly visible hemorrhage. (From Gaydos,<sup>111</sup> courtesy of the author and New England Journal of Medicine.)

In acute leukemia or when thrombocytopenia is induced by chemotherapy, decreased platelet production almost always is responsible for the reduction in blood platelets. Examination of bone marrow from such patients reveals decreased numbers of megakaryocytes. Some shortening of platelet survival may also contribute to thrombocytopenia.<sup>145a</sup>

Decreased production and shortened intravascular survival both contribute to thrombocytopenia in patients with advanced HD.<sup>137</sup> and reduced survival has been found at diagnosis, even in the absence of thrombocytopenia.<sup>138</sup> Increased platelet destruction, presumably as a result of formation of platelet autoantibodies, is a cause of thrombocytopenia in an occasional patient with CLL.<sup>2,117</sup> In this instance, abundant megakaryocytes are present in the bone marrow in the face of severe thrombocytopenia, and platelet survival is markedly shortened. We have observed a similar picture in a patient with MM and it has been reported in patients with HD.<sup>6</sup> Platelet pooling in an enlarged spleen has been demonstrated as a contributing cause of thrombocytopenia in a few patients.<sup>137</sup> An average of 70% of platelets were

in the spleen in one series of patients with HD, even though thrombocytopenia was not present.<sup>138</sup>

*Therapy of thrombocytopenic bleeding depends, in part, upon the cause of the thrombocytopenia. If severe bleeding develops, prompt and vigorous use of platelet transfusion is indicated.*<sup>146,156,174,175</sup> The usefulness of platelet transfusion in the treatment of thrombocytopenic hemorrhage was demonstrated in a comparative study of the transfusion of fresh versus stored blood.<sup>149</sup> Transfusion of platelet substitutes or of nonviable platelets was proved to be ineffective.<sup>145</sup> Transfusion of freshly prepared platelet concentrates is the therapy of choice.

Techniques for obtaining, storing, and transfusing isologous platelets were discussed in Chapter 12. With hemorrhage, the platelets from at least 8 units of blood should be given to an adult and those from at least 4 units to a child. No upper limit for the number of platelet transfusions can be stated since vigorous administration should be continued until bleeding has been interrupted.

If the picture is compatible with increased platelet destruction, a situation seen most often in CLL, as mentioned above, therapy



with adrenal steroids is recommended. Initially at least 1 mg/kg of prednisone or its equivalent should be given. If platelet destruction is not interrupted by steroids or if large doses must be used over a prolonged period, eg, more than 10 mg/day of prednisone for more than six months, splenectomy should be considered.

The only effective long-term treatment for thrombocytopenia secondary to decreased platelet production is to induce significant improvement in the underlying disease or to discontinue antitumor therapy if that is thought to be the cause. Thrombocytopenia remits as remission is induced in acute leukemia<sup>1</sup> or if marrow function is improved by therapy in other diseases such as HD.<sup>161</sup>

*Prophylactic platelet transfusions* for severely thrombocytopenic patients with neoplastic disease are commonly used. Their effect in reducing the frequency of death due to hemorrhage seems unquestioned (Chapter 47, Table 47-4). However, in many instances the cost is prohibitive. Platelet transfusion commonly fails in two circumstances: in the febrile infected patient and in the patient who develops isoantibodies to platelets.<sup>174,178</sup> In both circumstances, platelet utilization is so brisk that increased counts cannot be maintained by transfusion.

Since the risk of fatal hemorrhage is minimal with a platelet count above  $20 \times 10^9/l$  (Fig. 54-6), this value is often used as the minimal desirable one in a prophylactic transfusion program. In the absence of infection, transfusion twice a week with the platelets from 6 to 8 units of blood usually will keep the platelet count above  $20 \times 10^9/l$ . However, isoantibodies often develop within a few weeks<sup>175,176a</sup> unless a single donor, preferably an HLA-compatible sibling or an unrelated but HLA-compatible donor,<sup>179</sup> is utilized. The development of isoantibodies is evidenced initially by failure to raise the platelet count by transfusion, an event that can also reflect incorrect processing of the platelets (Chapter 12). Thus, failure to increase the count on more than one occasion should be documented before assuming that isoantibodies have developed.

A prophylactic transfusion program is difficult to justify in all severely thrombocytopenic patients with neoplastic disease. In the patient in whom an improved platelet level can be anticipated following therapy, or when thrombocytopenia is the result of therapy and therefore the condition is expected to improve shortly, such a program has potential value. One alternative to the prophylactic use of platelets is to employ platelet transfusions to interrupt bleeding once it has begun. The difficulty with this approach is that subarachnoid hemorrhage is a common cause of death from thrombocytopenia, especially in AML patients,<sup>1</sup> and transfusion, once subarachnoid bleeding begins, is rarely of benefit. Secondly, interruption of severe hemorrhage may require more platelets than are immediately available since they are rapidly utilized until bleeding has been interrupted. Thus, we prefer a prophylactic transfusion program for patients in whom there is reasonable expectation for improved platelet production within a few weeks. However, if such expectation is unrealistic, as in the patient with AML who has failed to respond or is no longer responsive to useful forms of therapy, prophylactic platelet transfusion is likely to be futile. It may change the cause of death, but will not prolong useful life to any significant degree.

### Disseminated Intravascular Coagulation

Disseminated intravascular coagulation (DIC) (Chapter 38), with significant hemorrhage, may occur during infection, especially with gram-negative sepsis.<sup>149</sup> In addition, a DIC-like syndrome complicates certain cases of acute promyelocytic leukemia and, rarely, other forms of AL in the absence of infection.<sup>178</sup> Surveys of leukemia populations reveal modest decreases in fibrinogen in the absence of hemorrhage in some patients.<sup>153,177</sup> Although this pattern was thought initially to be the result of increased fibrinolysis, fibrinolysis as such usually cannot be documented and it appears more likely that most, if not all, cases represent instances of DIC.<sup>179,148,153,158,162,171</sup> However, modest

degrees of fibrinolysis may contribute to thrombocytopenic hemorrhage in the absence of DIC in occasional leukemic patients<sup>167</sup> and high levels of fibrinolysins have been noted in patients with CLL.<sup>141,168</sup>

*Promyelocytic leukemia* is often associated with bleeding. Then hypofibrinogenemia as well as thrombocytopenia and often reduction of other clotting factors are seen. This pattern is encountered on rare occasions in patients with acute monocytic,<sup>1,142,162</sup> myeloblastic,<sup>164</sup> or lymphoblastic leukemia<sup>148</sup> and even in NHL.<sup>166</sup> In these patients, very large, rapidly spreading ecchymoses are found. In the typical patient with this syndrome the promyelocytes are characterized by numerous, abnormally large, eosinophilic-staining granules and often some basophilic granules and other crystalline inclusions are present as well.<sup>143,173,176</sup> Large amounts of thromboplastic activity in extracts from such cells have been demonstrated in vitro, suggesting that DIC may be triggered by a product of the leukemic cell.<sup>142,163,170</sup>

Therapy of patients with this form of bleeding, as of those with most complications of acute leukemia, is of transient benefit unless the underlying disease can be modified. Although certain authors have suggested that induction of remission in such patients is quite difficult,<sup>143</sup> we have observed the same rate of remission as with other forms of AML.<sup>140</sup> The use of fibrinogen or of antifibrinolytic agents has been of questionable value.<sup>2,139,148,153,171</sup> A reasonable approach to the treatment of the patient with AL who has severe DIC as part of his initial picture is to institute intensive antileukemic therapy with the object of reducing the total number of leukemic cells as quickly as possible, since these are the presumed inciting cause of the DIC. If infection is present, intensive therapy for this complication should also be instituted promptly. Heparin should probably be given as well,<sup>153a</sup> and if severe thrombocytopenia and/or hypofibrinogenemia are present, replacement therapy should be given as heparin is administered.

*Vascular lesions* reminiscent of those seen in thrombotic thrombocytopenic purpura

(Chapter 34) have been observed in patients with acute leukemia and in patients with CML.<sup>160</sup> Whether these are simply the vascular lesions of DIC or are distinct will require further study.

*Thrombocytopathic hemorrhage* is observed in some patients with CML and AML<sup>144,145a</sup> as well as in patients with polycythemia vera (Chapter 30), myelofibrosis or idiopathic thrombocythemia (Chapter 57). As discussed in Chapter 57, this seeming paradox of bleeding in the presence of excessive numbers of platelets<sup>155</sup> is thought to be due to functional defects of platelets. Evidence for functionally deficient platelets has been presented in occasional patients with acute leukemia.<sup>145a,168</sup>

### Other Causes of Hemorrhage

*Liver disease* may result in hemorrhage, because of reduction in factors II, V, VII, IX, and X as well as other defects (Chapter 38). While significant liver disease may be the result of tumor infiltration in any of these conditions, it is more often due to complicating factors such as viral hepatitis or hepatotoxicity from antitumor therapy (Chapter 55). In two patients with acute leukemia, isolated and unexplained severe deficiency of factor V and of factor X, respectively, were considered to be the cause of hemorrhage.<sup>152</sup> In another case, a circulating anticoagulant, apparently produced by leukocytes, was thought to be the cause of hemorrhage in a patient with AML.<sup>156</sup> We have also observed circulating anticoagulants of unknown sources in a patient with MM. Patients with paraproteins in high concentration may have hemorrhagic manifestations due to the hyperviscosity syndrome (Chapter 52). Chronic subdural hematoma, perhaps secondary to meningeal leukemia, has been observed in ALL.<sup>168a</sup>

### Abnormalities of Erythrocytes

#### Anemia

Anemia is found in all of the hematologic malignant diseases (Chapters 46-53) and in

some, such as the acute leukemias and myeloma, it is such a common part of the clinical picture that one must be particularly cautious in making a diagnosis in its absence. Anemia is responsible for some of the complaints of *fatigue* and a *vague sense of ill health*. In general, the presence and severity of anemia usually reflect the severity of the disease.

The anemia is most often normochromic and normocytic, although in certain circumstances it may be macrocytic or marked anisocytosis and poikilocytosis may be present. The primary mechanism of anemia, in most instances, is *decreased production of red cells*, reflected in decreased reticulocytes and decreased nucleated red cells in the bone marrow. Iron kinetic studies indicate erythroid hypoplasia.<sup>219,221</sup>

The cause of the decreased red cell production is unknown. It is often attributed to replacement of the marrow by tumor, but there are reasons for thinking that simple mechanical displacement of erythroblasts by tumor cells or competition for nutrients cannot explain decreased production in most patients. Many patients with lymphoma are anemic, but do not have demonstrable invasion of the marrow. In the leukemias, when marrow invasion is present, there is little or no correlation between the degree of anemia, thrombocytopenia, and neutropenia.<sup>1</sup> If "crowding out" were the primary problem, all normal cells would be expected to be equally affected. As in the anemias of chronic disorders (Chapter 18), transferrin levels tend to be decreased in most patients with hematologic malignant disease and serum iron levels are often low in those with CLL or MM.<sup>192,199,221</sup> On the other hand, serum iron levels usually are normal or elevated in AL and CML patients<sup>185</sup> and may be high, normal, or low in those with the lymphomas.<sup>219</sup> Thus, a high percentage of transferrin is saturated in many patients, and in this way the anemia differs from that of certain other chronic disorders (Chapter 18).

Erythropoiesis usually is under the control of erythropoietin in the leukemias and lymphomas,<sup>190,221</sup> even when the presumably malignant erythropoiesis of DiGuglielmo's

syndrome is present.<sup>198,201,215</sup> Erythropoietin levels are usually, but not invariably, increased to a degree appropriate to the degree of anemia.<sup>201</sup> Transfusion to a normal level of erythrocytes appropriately reduces the rate of red cell production to a degree corresponding to decreasing erythropoietin production. Interestingly, however, in two patients with CML in whom an erythroblastic crisis occurred, erythropoiesis that was autonomous of erythropoietin was noted.<sup>215</sup>

*Hemolytic anemia* of severe degree, associated with a positive reaction to Coombs' test, complicates the course of from 10 to 20% of patients with CLL and occurs in some patients with HD and NHL and occasionally in those with MM. Autoimmune hemolytic anemia may antedate the appearance of CLL, NHL, or HD by months<sup>218</sup> or years.<sup>191,202</sup> An episode of idiopathic autoimmune hemolytic anemia may be the first sign of leukemia or lymphoma. Severe hemolysis has been reported in a few CML and AML patients. In such subjects, while a positive Coombs' reaction has been noted,<sup>206</sup> the reaction more often has been negative.<sup>192,197</sup> In CLL there is no apparent relationship between the development of hemolysis and the severity or duration of the disease.<sup>2</sup> The antibody usually is more active in the warm than in the cold. Severe hemolysis as a primary cause of anemia probably does not occur in more than 3% of patients with HD.<sup>193</sup> Most of these patients have had fairly widespread disease but no special features predisposing to development of autoimmune hemolysis were detected and the hemolysis did not seem necessarily to be a bad prognostic sign.<sup>195,209</sup> As with CLL, the antibody usually is warm reacting and cold agglutinins usually are absent.<sup>195</sup> Thrombocytopenia, possibly of autoimmune nature, may be present as well.<sup>195</sup> The onset is often quite sudden and hemolysis can be exceedingly severe, so much so that death from cardiac failure has occurred before transfusions could be given.<sup>1,184</sup>

The diagnosis is established by the same studies that detect any form of hemolysis (Chapter 20), the indirect fraction of serum bilirubin usually is elevated, and excretion of

urobilinogen in urine and feces is abnormally high. Often there is an appropriate marrow response reflected in increased blood reticulocytes, but this may be absent, particularly if the patient is receiving cytotoxic chemotherapy when hemolysis begins.

Less severe hemolysis, reflected only in a modest decrease in red cell survival without reticulocytosis or hyperbilirubinemia, may occur.<sup>192,203,208,217</sup> When red cell survival is shortened in the leukemias, lymphomas, or myeloma, the reduction is modest, rarely exceeding a two-fold decrease unless autoimmune hemolytic anemia is present.<sup>203,221</sup> Sequestration of erythrocytes with or without increased red cell destruction in large spleens may contribute to the anemia.<sup>187</sup>

A degree of *ineffective erythropoiesis* is found in some patients, most strikingly in those with DiGuglielmo's syndrome, as determined by percent incorporation of <sup>59</sup>Fe into circulating red cells or by a discrepancy between plasma iron turnover and reticulocyte numbers,<sup>193,201,215</sup> but iron utilization appears to be normal in most patients with HD or NHL.<sup>199</sup>

*Macrocytic anemia with marrow cells resembling megaloblasts* is noted in patients with DiGuglielmo's syndrome and sometimes is found in other patients with AML in whom the predominant cell is a myeloblast rather than the proerythroblast of DiGuglielmo's syndrome. Megaloblastosis may result from the use of any of the antitumor agents whose primary effect is inhibition of DNA synthesis (Chapter 55), such as the folic acid antagonists, cytosine arabinoside, 6-mercaptopurine, and procarbazine. However, the abnormal nucleated red cells that may appear in AML in the absence of such drugs or occur as part of the terminal phase of CML or even polycythemia vera (Chapter 30) or myelofibrosis (Chapter 57) have certain morphologic features that often allow their differentiation from megaloblasts due to deficiency of B<sub>12</sub> or folic acid. Thus, the nuclear chromatin is not as fine as in true megaloblasts and a greater proportion of the erythroid precursors are more immature in most patients with DiGuglielmo's syndrome than in

those with B<sub>12</sub> or folate deficiency. Furthermore, in many patients with DiGuglielmo's syndrome, bi- and even tri-nucleated erythroblasts are much more numerous than in patients with B<sub>12</sub> and folate deficiency. In addition, the changes in neutrophils characteristic of those vitamin deficiencies, such as hypersegmentation, giant metamyelocytes, and macropolycytes, are usually absent.

*Nucleated red blood cells (NRBC)* are found easily in blood smears of most patients with DiGuglielmo's syndrome and may be more frequent than leukocytes. They may be found on blood smears of patients with other morphologic types of AML, but they are fewer in patients with ALL.<sup>1</sup> In CML, NRBC are found in blood smears but they are rarely as frequent as in myelofibrosis (Chapter 57). In CLL and in MM their presence usually indicates complicating factors such as hemorrhage or hemolytic anemia. Marrow invasion or myelofibrosis associated with HD or NHL often is associated with the presence of NRBC on blood smears.

*Therapy for the usual anemia* that is present in leukemia, lymphoma, and myeloma patients may be difficult. Transfusions should be used when required for control of symptoms and signs due to the anemia (Chapter 13). Appropriate studies may be indicated to rule out the possibility of deficiency of iron, B<sub>12</sub>, or folic acid as a cause of the anemia, but therapy with these agents as a routine procedure is of no benefit. In many cases, if the underlying disease can be alleviated by specific therapy, the anemia will be corrected or at least lessened. Thus, induction of remission or significant improvement in patients with acute leukemia, CML, the lymphomas, or myeloma leads to improved red cell production. However, this is not always the case in those with CLL.<sup>2</sup> Large doses of synthetic androgens are occasionally helpful in treating the anemia, as they are in treating aplastic anemia (Chapter 56). Steroids or splenectomy may lead to reduction in anemia in patients with CLL, even in the absence of overt hemolysis.<sup>2,187</sup>

*Therapy of autoimmune hemolytic anemia* consists primarily of transfusion, steroid ad-

ministration, and, in certain subjects, splenectomy, as discussed in Chapter 27. Prednisone, 40 to 60 mg/day, or other adrenal corticosteroid hormones in equivalent dosage, will interrupt or at least reduce the degree of hemolysis in most patients.<sup>2,195</sup> Use of the drug should be continued at full doses until all evidence of hemolysis has disappeared and the hematocrit has returned to prehemolysis levels. The dosage is then diminished gradually. In some patients, hemolysis does not recur as steroid administration is tapered and stopped. In most but not all patients in whom hemolysis recurs or in whom steroids failed to interrupt hemolysis, splenectomy controls the hemolytic process. If the patient is in otherwise relatively good condition, the modest risk of splenectomy<sup>188</sup> is preferable to the effects of long-term steroid therapy. Irradiation to the spleen has also been of benefit in patients with CLL in whom severe hemolysis failed to respond to steroid therapy, particularly in those in whom the reaction to Coombs' test was negative.<sup>193</sup> Splenic irradiation has also been of benefit in treating hemolysis in HD patients.<sup>195</sup> Spontaneous remission of hemolysis may occur.<sup>195</sup> The role of systemic antitumor therapy in treating hemolytic episodes is somewhat controversial. It has been suggested that hemolysis may be initiated by chemotherapy in CLL patients,<sup>201</sup> but decreased hemolysis has been reported following HN2 or irradiation of nodes in patients with HD.<sup>195</sup>

Fetal hemoglobin levels are abnormally high in some patients with leukemia. The highest levels, up to 76% Hb F, occur in patients with the infantile form of CML<sup>207</sup> (see Chapter 48) and in patients with DiGuglielmo's syndrome,<sup>181,186,200</sup> but modest elevation has also been observed in ALL<sup>182,207</sup> and AML patients.<sup>181,213</sup> Abnormally high levels have not been observed in patients with CLL or typical CML.<sup>181</sup> High levels may persist during complete remission in those with ALL.<sup>207</sup> The significance of the elevated Hb F remains to be determined, but its presence has led to the postulate that some forms of leukemia may involve a fetal rather than an adult stem cell.<sup>200</sup>

Hb A<sub>2</sub> has been found increased in some ALL and CML patients<sup>182</sup> and decreased in others.<sup>183</sup> Increased or decreased levels also have been reported in patients with DiGuglielmo's syndrome.<sup>183</sup> A fast A<sub>2</sub> component<sup>182</sup> and an unstable hemoglobin, probably distinct from Hb H,<sup>220</sup> have been noted.

*Red cell antigens* may be altered in some patients with acute or chronic myelocytic leukemia. This may take the form of loss of antigenicity to any isoagglutinins,<sup>190</sup> acquisition of new red cell antigens, usually A,<sup>180,210</sup> or changes in Rh grouping.<sup>216</sup> Loss of B antigen has been reported in CLL patients.<sup>211</sup>

## Complications Due to Infiltration of Organs

The frequency with which various organs are found to be infiltrated with tumor cells at autopsy<sup>3</sup> far exceeds the frequency of symptomatic complications associated with such infiltration. Differential diagnosis of complications due to infiltration, infection, or hemorrhage often presents a problem.

As a general rule, it can be stated that organ infiltration with the cells involved in leukemia, lymphoma, myeloma, and related diseases is less likely to interfere with the normal function of the infiltrated organ than when carcinomatous cells invade an organ. Thus, marked enlargement of such organs as liver and kidneys may not be accompanied by significant functional impairment.

Therapy of specific organ infiltration consists of systemic chemotherapy, local chemotherapy for certain skin lesions (page 1688), local irradiation of the involved area or surgical excision in certain selected circumstances, most notably in patients with spinal cord compression (page 1675), or the use of combined therapeutic modalities.

Therapy of extranodal infiltration given with the intent of curing the disease is limited to patients with lymphoma in a single extranodal site as, for example, the bowel (page 1682) or bladder (page 1684). In such persons the lesion usually is excised surgically for diagnostic purposes; apparent cures have followed simple surgical excision (see Chapter

51). However, it is generally recommended that excision be followed by irradiation therapy or that radiation therapy be used instead of excision if the latter was not given at the time of biopsy. Delivery of at least 3500 rads to the area of the lesion is recommended.

Palliative therapy of specific organ infiltration must be individualized, keeping in mind factors such as the extent of disease, its known responsiveness to various types of therapy, and whether or not significant symptoms or signs are the direct result of such invasion. In most instances, widespread disease is present and systemic chemotherapy is the treatment of choice. All of the tumors considered here usually are radiosensitive (Chapter 55); when reduction of a specific infiltration is urgent, local irradiation can be given in addition to chemotherapy. In some circumstances, such as in patients with CLL in whom chemotherapy may do more harm than good, or when isolated infiltrates occur in patients with acute leukemia who are otherwise in complete remission, the infiltrate may be treated with radiation therapy alone. A dose of 1000 to 2000 rads, delivered to the local lesion, usually is sufficient to accomplish palliation. Specific recommendations for therapy of specific lesions will be discussed when appropriate.

### Neurologic Involvement

The central nervous system, spinal cord, and cranial and peripheral nerves may be involved by infiltrating or compressing tumors, and symptoms may result.

#### Meningeal Infiltration<sup>273,282,283a,326,357</sup>

Patients with ALL often suffer from *diffuse* infiltration of the leptomeninges, producing what may be termed the *meningeal syndrome*. The frequency of this syndrome in different series of patients with ALL has ranged from 26<sup>282</sup> to 80%.<sup>283a</sup> The highest frequency is in recently reported series,<sup>283a</sup> and this apparent increase probably represents more frequent recognition of this complication as well as a true increase in incidence because of the lengthening life span of patients with ALL.

Meningeal infiltration may be present at diagnosis, and can develop at any time during the course of the disease. In one series<sup>253</sup> there was an accrual rate of the meningeal syndrome of 3.8% per month during the first year after diagnosis, decreasing to 2% per month during the second and third years. This syndrome has been observed in AML, but with less than half the frequency of ALL.<sup>253,282,357</sup> Its lesser frequency in AML may merely reflect the fact that remissions are less frequent and usually of shorter duration than in ALL. It is occasionally observed in patients with CLL, HD, NHL, or MM. In the last three, *patchy* tumor formation with local rather than diffuse CNS infiltration is more common than diffuse involvement.

The cerebral leptomeninges are infiltrated with lymphoblasts in a diffuse fashion and the infiltrate may extend to involve spinal meninges and the sheaths of cranial nerves. Perivascular infiltration of meningeal vessels usually is present and, although some perivascular infiltration of intracerebral vessels may also be found, frank intracerebral tumor formation is not commonly associated.

The signs and symptoms characterizing the syndrome are summarized in Table 54-3. They are primarily those of increased intracranial pressure. Headache, lethargy, nausea, and vomiting are the cardinal manifestations. Unless the patient is examined early in the evolution of the syndrome, papilledema, stiff neck, and positive Kernig and Brudzinski signs usually will be found. In young children, spreading of sutures may be observed (Fig. 54-7). Grand mal seizures may occur. However, if a high index of suspicion is maintained, a diagnosis of the meningeal syndrome often can be made when headache is the only symptom and when no abnormal signs are present. Examination of the CSF at the time of diagnosis revealed evidence of meningeal leukemia in four of 47 children with ALL in whom no symptoms or signs suggesting the syndrome were present.<sup>318</sup>

*Cranial nerve palsy* accompanies approximately 20% of meningeal syndromes. The seventh nerve is most often affected, closely followed by the sixth and third, but any nerve

Table 54-3. Signs and Symptoms of CNS Involvement Occurring in 59 Patients\*

Secondary to Increased CSF Pressure (56 Pts)		Psychic Disturbances (12 Pts)	
Vomiting	46	Hyperirritability	8
Headache	45	Hallucinations	3
Papilledema	34	Catatonic depression	1
Lethargy	22	Disorientation	1
Vertigo	9	Cushing's Syndrome (8 Pts)	
Nuchal rigidity	7	Pathologic weight gain	8
Convulsions	4	Hirsutism	1
Coma	3	Auditory Disturbances (5 Pts)	
Proptosis	3	Hypacusis	4
Hydrocephalus	1	Hyperacusis	2
Ocular disturbances (21 Pts)		Autonomic Nervous System Dysfunction (5 Pts)	
Diplopia	9	Hyperpnea	3
Strabismus	9	Cheyne-Stokes respiration	2
Blurred vision	8	Fever	1
Blindness	3	Hiccough	1
Photophobia	3	Tachycardia	1
Nystagmus	3	Speech Disturbances (3 Pts)	
Other visual disturbances	2	Slurred speech	2
Cranial and Peripheral Nerve Dysfunction (13 Pts)		Hoarse voice	1
Facial paralysis	6	Asymptomatic Episodes (3 Pts)	
Hemiparesis	3	Dx by CSF	2
Paraplegia	3	Dx at autopsy	1
Foot drop	1		
Ptosis eyelid	1		

\*From Hyman et al.<sup>252</sup> courtesy of the authors and Henry M. Stratton, Inc

may be involved. Localized cranial nerve involvement may occur in the absence of the meningeal syndrome (page 1675).



Fig 54-7 Widening of cranial sutures as a consequence of meningeal leukemia (From Shaw et al.<sup>357</sup> courtesy of the authors and Neurology)

Hyperphagia and obesity occasionally develop concurrently with the meningeal syndrome and are thought to reflect leukemic infiltration of the medioventral hypothalamic nuclei.<sup>221</sup>

The diagnosis is made by examination of spinal fluid. Lymphoblasts or myeloblasts in spinal fluid may be difficult to identify in routine preparations. Special means of obtaining good morphologic preparations, such as making a thin cellular preparation on plastic filters,<sup>3,36</sup> resuspending concentrated cells from spinal fluid in serum and making thin smears,<sup>361</sup> or utilizing a specially designed centrifuge for preparing smears (cyto-centrifuge),<sup>283a</sup> allow for more definitive morphologic identification than do routine methods. No increase in the number of other types of cells should be found in the spinal fluid. Rarely, no increase in the number of cells is observed.<sup>273,357</sup> The opening pressure

usually is elevated although it may not be increased early in the course of the syndrome. The protein content usually is increased and the sugar content is decreased in inverse relation to the degree of spinal fluid pleocytosis. Some investigators accept 10 mononuclear or blastic cells/ml in the absence of an identifiable organism to be sufficient evidence for diagnosis.<sup>365</sup> Still others<sup>283a</sup> consider any identifiable leukemic cells in spinal fluid to be sufficient.

The hazard of lumbar puncture with increased spinal fluid pressure does not appear to be as great in the meningeal syndrome as it is, for example, in various forms of brain tumor. Among at least 500 lumbar punctures in patients with the meningeal syndrome personally observed, herniation of the uncus occurred only once. There is a slight, but definite, hazard in doing lumbar puncture in thrombocytopenic patients. One of our patients suffered hemorrhage into the spinal canal sufficient to cause paralysis of the cauda equina. However, the need to make an accurate, prompt diagnosis of the cause of cerebral symptoms and signs in patients with leukemia outweighs the slight risk of lumbar puncture.

The differential diagnosis includes subarachnoid hemorrhage, intracerebral infiltration, and bacterial, fungal, and viral meningitis or encephalitis. The first two usually are accompanied by rapid onset of severe, localized neurologic signs, which are rare in the meningeal syndrome. Culture of spinal fluid and the presence of neutrophils and microorganisms on microscopic examination usually allow prompt recognition of bacterial meningitis as distinguished from the meningeal syndrome, and culture and India-ink preparations serve to identify fungal infection. Viral encephalitis is the most difficult differential diagnosis and it is here that good preparations for cellular identification<sup>297</sup> become critical. Lymphoblasts or myeloblasts indicate the meningeal syndrome; they are not likely to be present with any type of infection. Occasionally the meningeal syndrome and infectious meningitis coexist and then a mixed cellular picture is expected.

Meningeal infiltration often occurs in patients with ALL or AML who are otherwise in complete remission, as may other extramedullary infiltrates.<sup>355</sup> If this complication is going to develop, it usually appears during the first two years of disease (median time of onset one year).<sup>253,283a</sup> The development of the meningeal syndrome during complete remission is disconcerting; it is not known whether it develops from residual leukemic cells that were in the meninges when remission developed, from ingress of leukemic cells during "remission," or from leukemic cells newly formed in the meninges. Since none of the antitumor drugs used in remission maintenance crosses the blood-brain barrier in a concentration sufficient to kill leukemic cells, this has been suggested as the primary factor in the development of the syndrome during remission. However, the demonstration during remission of infiltrates in other areas such as the testis, ovaries, and kidneys,<sup>355</sup> which are not protected from drug exposure, throws some doubt on this hypothesis.

Although the development of the meningeal syndrome may be the first evidence of general relapse from remission, in and of itself it is not necessarily a bad prognostic sign if proper treatment is given.

### Treatment

Treatment of patients with the meningeal syndrome can be carried out with systemic drugs, steroids if the leukemia is still steroid responsive,<sup>357</sup> pyrimethamine<sup>262</sup> or BCNU,<sup>285</sup> by intrathecal installation of aminopterin,<sup>348</sup> methotrexate,<sup>465</sup> or cytosine arabinoside,<sup>376</sup> or by x-irradiation of the entire meninges. Entirely satisfactory management can be accomplished with intrathecal methotrexate (MTX) and in certain selected circumstances by whole brain irradiation. Aminopterin may have had a slightly superior ratio of therapeutic to toxic effect than MTX,<sup>348</sup> but the former drug is no longer available. Intrathecal MTX is effective even when systemic resistance to the drug has developed.<sup>365</sup> The concentration of the drug



that is achieved in spinal fluid is such that the increased dihydrofolate reductase of the MTX-resistant cell (see Chapter 55) fails to protect the cell against the drug. Considering the effectiveness of MTX, the wealth of experience with this form of therapy, and the rarity of serious toxicity accompanying its use (see below) there seems little reason to use other available forms of intrathecal therapy.

*Methotrexate*, 0.5 mg/kg, not to exceed a total dose of 20 mg, in a solution of sterile saline, is injected through the lumbar puncture needle (LP) after withdrawal of a similar or greater volume of CSF. For adequate distribution of the drug to occur, the volume of injected material is important and should exceed 10% of the CSF volume; thus, 10 to 20 ml of solution should be injected.<sup>347</sup> If the patient is kept in bed with the foot of the bed slightly elevated for an hour or two, the rate of diffusion into the cerebral space may be enhanced.<sup>347</sup> Some abatement of symptoms may be apparent immediately, presumably as the result of reduction of CSF volume and pressure.<sup>357</sup> Dexamethasone may produce prompt symptomatic relief, even in patients whose disease is no longer steroid responsive, presumably by reversing cerebral edema since neither reduced pressure nor fewer cells necessarily follow its use.<sup>321</sup> It is our practice to instill MTX with the initial diagnostic LP in any patient suspected of having the meningeal syndrome. If the diagnosis is incorrect, little harm has been done and, if correct, one additional LP for initial MTX instillation has been avoided. Lumbar puncture with MTX instillation is done twice each week until the pressure has been normalized and all leukemic cells have disappeared from the fluid. The protein concentration may remain elevated for some time after the cells have disappeared. Diffusion of MTX from the CSF is slow; treatment twice a week usually assures an effective CSF level at all times.<sup>345</sup>

Reports of serious complications relating to intrathecal instillation of MTX are few and consist of cases of *arachnoiditis*<sup>305,365</sup> and possible cases of fatal cerebral demyelination<sup>365</sup> and encephalopathy.<sup>291</sup> However, im-

mediate, transient symptoms of headache, fever, and/or vomiting were noted in 38% of patients receiving the drug for prophylactic therapy (see below); these symptoms became more severe after repeated injections had been given.<sup>366</sup> Since the slow diffusion of MTX from CSF to circulation leads to significant effects on bone marrow, the use of other drugs should be discontinued or at least their dosage should be reduced during therapy unless *citrovorum* factor is given to counteract systemic toxicity.<sup>365</sup>

Delivery of at least 1000 rads to the cerebral and spinal meninges also is quite effective.<sup>365</sup> Lower doses of *irradiation*, or omission of spinal irradiation when the skull is irradiated, either fail to correct pleocytosis or do so only transiently.<sup>365</sup> However, because of the bone marrow depression and transient alopecia associated with x-ray therapy, such therapy is usually employed only for prophylactic purposes (see below) or in a few patients who respond poorly to MTX. With any evidence of intracerebral lesions, response to intrathecal therapy is not expected to be particularly beneficial and irradiation or irradiation together with methotrexate should be employed.<sup>273,365</sup>

Prophylactic therapy of the meningeal syndrome has been shown to be feasible, at least in some patients with ALL. This topic has been reviewed in depth.<sup>283a</sup> The frequency with which the meningeal syndrome develops during initially induced remissions in children with ALL can be reduced as much as 13-fold (62% to 5%) by prophylactic therapy. Craniospinal irradiation or cranial irradiation plus repeated intrathecal MTX appear to be equally effective and are the treatments of choice. After the patient has been brought into remission by chemotherapy, CNS prophylaxis is begun. With craniospinal irradiation, 2400 rads are delivered in 24 days. In children age one to two years, the dose is reduced to 2000 rads and in those less than one year of age to 1500 rads. With cranial irradiation plus MTX, 2400 rads (or less, according to age, as described above) are given in 18 days and MTX, 12 mg/m<sup>2</sup>, is given intrathecally every three days for a

total of five injections. Lower doses of irradiation, 500 rads or 1200 rads, have little or no prophylactic effect. Intrathecal MTX without irradiation, either as a short, intensive initial course or given at spaced intervals throughout remission, has had some prophylactic effect but probably is inferior to the above-described forms of therapy. BCNU, a drug that crosses the blood-brain barrier (Chapter 55), had no apparent effect in preventing meningeal leukemia when given during remission.<sup>366</sup> Intensive systemic chemotherapy during remission may in and of itself reduce the frequency of meningeal leukemia.<sup>283a</sup>

Prophylactic regimens may have toxic effects.<sup>283a</sup> Bone marrow depression is observed, seemingly more severe with cranio-spinal irradiation than with cranial irradiation plus MTX. Approximately 10% of patients given either regimen have developed a syndrome, some five to seven weeks following radiotherapy, which is self-limited but of unknown cause and consists of fever, somnolence, dizziness, and CSF pleocytosis. Irradiation was followed by gliosis and neural degeneration in one patient, with spastic quadriplegia and mental deterioration. One patient has developed NHL of the basal ganglia and another developed CML, but whether such complications are the result of CNS therapy or of intensive chemotherapy has not been determined.

Prophylactic CNS therapy is not employed by all investigators. Since a number of patients do not develop the syndrome, they believe that such treatment is unnecessary and causes alopecia and the morbidity associated with repeated lumbar puncture and MTX installations.<sup>366</sup> However, once the syndrome has developed, and particularly if there are recurrent episodes, prophylactic therapy should be given and in such patients we have employed irradiation as well as MTX.

Recurrence of the meningeal syndrome is expected after the first episode. On the average, symptoms are again manifest within three months of adequate treatment of the initial episode.<sup>263</sup> However, recurrence may be delayed and in a few patients the syndrome

may not develop again even though they live for three or more years. A "prophylactic" regimen may not be as effective after an episode of meningeal leukemia has developed and has been treated as when it is given at the onset of remission.<sup>283a</sup>

### Localized Involvement of the Brain

Intracerebral leukemia develops in association with a rapidly rising leukocyte count in as many as 20% of patients with AML and with lower frequency in patients with ALL or in the blastic crisis of CML.<sup>150,261,326</sup> The number of blasts in the blood usually increases exponentially when these lesions develop. Autopsy examination suggests the following sequence of events: leukostasis in small vessels due to a growing mass of leukemic cells; disruption of the vessel due to the expanding mass; massive hemorrhage surrounding the growing lesion as other vessels are disrupted by the mass. The intracerebral leukemic masses are usually spherical and may be as large as golf balls. The surrounding hemorrhage may destroy large areas of the brain and multiple masses usually are found (Fig. 54-8).

Intracerebral hemorrhage, usually associated with sudden death, may be the first sign of cerebral invasion, but more often premonitory signs of headache or the sudden development of localizing neurologic signs and symptoms are present. A rapidly rising leukocyte count should be considered an indication that intracerebral leukemia probably is developing.

Therapy must be directed toward reducing the leukocyte count as quickly as possible by vigorous chemotherapy. Once symptoms or signs of intracerebral leukemia have developed, therapy is rarely of any benefit and, indeed, the complication may continue to evolve even as leukocyte counts are reduced by therapy.

*Local tumors due to NHL*, particularly Burkitt's lymphoma<sup>366</sup> and histiocytic lymphoma, may develop intracerebrally, in the dura or meninges, or in bones of the skull



Fig 54-8 A Multiple intracerebral hemorrhagic lesions in a patient with a leukocyte count greater than  $300 \times 10^9/l$  at the time of death. B, Higher magnification of the lesion in 'a' to show distinctive appearance of confluent nodules of leukemic cells surrounded by hemorrhage. C, Microscopic appearance of this type of intracerebral lesion; hematoxylin and eosin  $\times 13$  (Illustration reduced to about 42% of original size) (From Moore et al<sup>328</sup> courtesy of the authors and Archives of Internal Medicine)

with local extension compressing the cerebrum.<sup>8,250</sup> This complication is quite rare in HD<sup>1,5,250</sup> or MM.<sup>313</sup> The tumor, like any other brain tumor, produces signs and symptoms depending upon its location. Complications, such as diabetes insipidus, have resulted from small tumors infiltrating the posterior pituitary gland.<sup>303</sup> The diagnosis may be difficult to establish with certainty unless surgical exploration with biopsy is undertaken. This is usually unwarranted as most such patients have widespread disease. Examination of CSF usually discloses a high protein content, but other findings are quite variable and identifiable tumor cells are found only rarely. The lesions may be located by brain scans or by cerebral angiography. In the usual case, if no other explanation for the CNS finding is uncovered, if the picture is not compatible with multifocal leukoencephalopathy (see below), and if there is nothing to suggest brain abscess, it is assumed that a hematologic tumor involving the brain is present.

Radiation to the demonstrated mass or to the whole brain if more than one mass is present or when a mass has not been located accurately is the treatment of choice. Since cure is not to be expected, 2000 rads usually are sufficient and rarely produce any complications other than hair loss. Steroids, procarbazine, and BCNU cross the blood-brain barrier and may have a favorable effect on tumors that are sensitive to these drugs

(Chapter 55). We have observed clearing of CNS signs and symptoms in a patient with HD treated with combination chemotherapy that included prednisone and procarbazine<sup>161</sup> (Chapter 50).

*Multifocal leukoencephalopathy* is a rare syndrome affecting some patients with ALL,<sup>230</sup> HD, NHL, CML, or CLL as well as those with chronic non-neoplastic diseases such as sarcoidosis or tuberculosis.<sup>345,377</sup> Evidence for a viral cause has been presented.<sup>377</sup> The complication usually develops in severely ill patients with widespread disease, but we have observed it in a patient with well-controlled CML.

Signs and symptoms are quite variable. Widespread but erratically distributed demyelination of the CNS is found at autopsy. Vision, motor function, and speech are most often affected and signs are usually bilateral. A steady progression of disease, commonly accompanied by dementia, with death occurring within one to six months of onset of symptoms is anticipated although survival for a year has been reported, as has apparent cure following therapy with cytarabine.<sup>229,345</sup> Examination of the CSF usually reveals no abnormality or only slight increase in protein, pressure, or number of lymphocytes. The EEG, while abnormal, is nonspecific, usually showing diffuse slowing in all leads. Brain scan, arteriography, or air studies may reveal cerebral atrophy, but do not yield a specific diagnosis. Features suggesting CNS tumors

rather than multifocal leukoencephalopathy are headache, seizures, single localized neurologic signs, and significant degrees of CSF abnormality.

*Spinal cord compression* is produced by tumor in the cord, the spinal dura, or meninges; or by vertebral tumor extending into the spinal canal and compressing the cord; or as a result of vertebral collapse and spinal cord compression. It is common in MM, affecting as many as 20% of patients<sup>3</sup>; it is present in 3 to 8% of HD patients.<sup>332,359</sup> It is less common in patients with NHL,<sup>359</sup> and is rare in those with leukemia although it can occur in association with any form of leukemia.<sup>389</sup>

There may be premonitory symptoms, such as paresthesias for days or weeks, but in some patients the interval between the first symptoms and complete paraplegia is measured in hours. Since recovery from paraplegia is unusual, any symptom suggesting cord involvement should lead to immediate complete evaluation. Therapy depends, in part, upon the severity of the signs and symptoms.<sup>332,359</sup> If severe paraparesis is evident, the myelogram should be obtained in the operating room and the area shown to be affected should be explored immediately. Postoperative irradiation therapy is advisable even when it is believed that most of the tumor has been removed surgically. If symptoms and signs have not progressed beyond the point of mild paresis, a trial of irradiation therapy may be given, but immediate laminectomy should be planned if any progression of signs occurs. Systemic chemotherapy should be given concurrently, especially when rapid response to therapy can be anticipated, as in HD.<sup>359</sup> Regression of mild paraparesis has followed chemotherapy alone in Burkitt's lymphoma.<sup>386</sup>

The urgency of therapy in this complication cannot be stressed too strongly. When treatment is delayed until the lesion has produced paraplegia, the latter almost always proves permanent, despite surgical and irradiation therapy.

*Cranial nerves* may be involved because of local infiltration after leaving the brain or as part of the meningeal syndrome (page 1669),

or tumor may involve their cerebral origins. The seventh nerve is most often affected peripherally, in which case a "peripheral" rather than a "central" facial palsy will ensue. Unilateral papilledema, proptosis, deafness, or vestibulatory disturbances are other findings that may be due to peripheral cranial nerve infiltration, and bilateral optic nerve infiltration, persisting after other manifestations of the meningeal syndrome had disappeared, has been observed.<sup>251a</sup> X-irradiation to the track of the nerve as it leaves the specific foramen of the skull is the treatment of choice.

*Peripheral nerves* are affected occasionally, usually due to compressing tumor masses. With HD or NHL, axillary or supraclavicular masses may cause pressure damage to nerves of the cervical plexus, and retroperitoneal nodes may compress lumbar or sacral nerve roots. Infiltration of dorsal root ganglia or of peripheral nerves themselves may occur with leukemia, lymphoma, or myeloma.<sup>247</sup> Peripheral neuropathy, with no apparent cause, has been reported.<sup>233,248</sup>

## Ocular Disease

Abnormalities of the orbit and of the globe or the nerves of the eye are rather frequent, although, in patients with HD, involvement of the globe is quite rare.<sup>223</sup> Fundic hemorrhage is common and usually is due to anemia,<sup>1</sup> although thrombocytopenia (page 1662) and leukemic infiltration in the retina<sup>223</sup> may contribute to this complication. Tumors of the orbit, displacing the globe and producing proptosis, may occur occasionally with any type of leukemia or lymphoma, but are most common in children with AML (*chloroma*) (Chapter 47). Cranial nerve involvement affecting the eye has been discussed above.

Although various portions of the globe may be infiltrated, the avascular cornea and lens are usually, but not invariably, spared this complication<sup>223</sup> (Fig. 54-9). Small infiltrates in the conjunctiva or the sclera, sometimes visible grossly, but usually asymptomatic, may be observed in patients with any

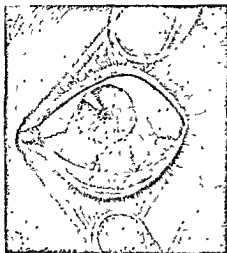


Fig 54-9 Lymphocytic tumor of cornea in patient with chronic lymphocytic leukemia

form of leukemia or lymphoma as well as in those with myeloma.<sup>223</sup> Choroidal and iris infiltration, most common in AL patients, are likewise usually asymptomatic but have resulted in such complications as glaucoma in ALL<sup>258</sup> and CLL patients<sup>269</sup>, lesions with the appearance of an hypopyon in AML and NHL patients<sup>293</sup>, and macular detachment in those with ALL.<sup>387</sup> X-irradiation of the orbit was beneficial in all of these patients.

Nodular or diffuse infiltration of the retina has been associated with various forms of leukemia or lymphoma,<sup>223,301</sup> and the vitreous body has been infiltrated in MM and AL subjects.<sup>223</sup> These infiltrates may be associated with areas of retinal necrosis, retinal detachment, and visual symptoms.<sup>223</sup> They may be seen with an ophthalmoscope. Increased tortuosity of the vessels<sup>320</sup> and microaneurysms<sup>251</sup> have been observed in the fundus of patients with CLL and of those with CML.<sup>251</sup>

#### Ears, Nose, Throat, Larynx, and Oral Cavity

The ears may be affected by hemorrhage,<sup>332</sup> infection (page 1656), or central nervous system or cranial nerve infiltration (pages 1669-1675) with hearing deficit, tinnitus,

pain, or vertigo resulting.<sup>353</sup> Symptoms due to infiltration of the structures of the inner ear are uncommon.<sup>306,351</sup>

The nasopharynx, pharynx, and larynx, when involved by infiltrative disease, usually are affected by involvement of Waldeyer's ring (adenoids, tonsils, and lymphoid tissues on the posterior pharyngeal wall and at the base of the tongue) by lymphoid neoplasms (see Chapters 47, 48, 49, 50, 51, and 52 and Fig. 51-3). Rhinophyma has been reported as being due to CLL.<sup>2</sup> Blockage of nasal passages due to AML infiltrates has occurred<sup>272</sup> and NHL of the nasal septum has produced a picture similar to that of idiopathic midline granuloma.<sup>286</sup> Laryngeal infiltration, severe enough to require tracheostomy, has occurred in patients with AML.<sup>289</sup>

The mouth and gums are rarely infiltrated except in persons with the monocytic variants of AML (Chapter 47).

Infiltration of the salivary glands producing reduced saliva and symptoms and signs reminiscent of Mikulicz's<sup>327</sup> or Sjögren's<sup>367</sup> syndromes has been observed in association with the acute leukemias<sup>1</sup> (Fig. 54-10).

#### The Lungs

*Infiltration of the pulmonary parenchyma* severe enough to be demonstrable in chest x rays is common in HD, is observed with some frequency in NHL, ALL, and AML, and during the blastic phase of CML, but is quite unusual in CLL or MM.<sup>2,6,9,296,333</sup> In as many as 10% of patients with HD there is pulmonary involvement at the time of diagnosis and probably at least half of those who are not cured eventually have this complication (see Chapter 50). In HD and NHL patients, pulmonary disease usually is the result of direct extension from disease in mediastinal or hilar lymph nodes, but lesions more distal and seemingly the result of blood-borne metastases also occur. In leukemia, association of the pulmonary infiltrate with disease in the hilum usually is not apparent.

Hodgkin's disease and NHL apparently limited to the lung and without associated hilar or mediastinal disease may occur.<sup>5,8</sup> It



Fig 54-10 Mikulicz's syndrome in a patient with chronic lymphocytic leukemia. Note the symmetrical swelling of the lacrimal and salivary glands. There was no leukocytosis ( $WBC\ 7.7 \times 10^9/l$ ), and only moderate lymphocytosis (lymphocytes 86%). The patient died of lobar pneumonia; the diagnosis was confirmed at autopsy. (From Jacobsen and Schaffer,<sup>285b</sup> courtesy of the authors and American Journal of Diseases of Children.)

has been suggested that certain lymphocytic tumors are not truly lymphosarcoma in that the eventual development of disseminated LSA after their surgical removal has been quite unusual as compared to those in which the pathologic appearance was that of RSCa.<sup>351</sup>

There is nothing diagnostic about the roentgenographic appearance of pulmonary infiltrates. For example, there may be single or multiple discrete rounded tumors of any size, massive invasion of a lobe, miliary lesions throughout the lung, or simply increased density of "pulmonary markings" in a part of or in all the lung. Cavitation of infiltrates has been observed in patients with HD<sup>363</sup> and AL.<sup>385</sup> Pulmonary infarction due to leukemic cells obstructing vessels may occur in patients with AL.<sup>285</sup> The lesions may be asymptomatic or may be associated with cough, chest pain, increased sputum production, and/or hemoptysis. Respiratory obstruction and atelectasis due to endobronchial HD have been noted.<sup>363</sup> Diffuse infiltrates may compromise lung function, pro-

ducing decreased lung volume, reduced carbon-monoxide diffusing capacity, and hypoxemia. Alveolocapillary block has resulted from infiltration in AL and CLL patients and respiratory distress was the presenting complaint of a patient with ALL in whom the alveolar walls were infiltrated.<sup>263</sup>

Differential diagnosis between tumor infiltration, infection (page 1656), and hemorrhage due to thrombocytopenia (page 1662) often is quite difficult. Pulmonary embolus with infarction can occur, even in thrombocytopenic patients.<sup>379</sup> Pulmonary alveolar proteinosis has complicated CML,<sup>249</sup> and hyaline membrane formation has been associated with HD.<sup>238</sup> Microscopic and cultural examination of the sputum for infecting organisms is important in the differential diagnosis. When uncertainty exists as to whether the lesion is due to tumor or infection it is usually wise to institute an appropriate therapeutic trial for infection. For instance, if there is any reason to suspect pulmonary tuberculosis, it is wise to institute INH therapy even if tuberculosis has not been proved. In certain

circumstances, biopsy proof of the diagnosis should be sought, as, for example, when endobronchial lesions accessible to bronchoscopy are present.<sup>363</sup> If the lesion is in the periphery of the lung, a needle biopsy may be attempted if blood coagulation is normal. Thoracotomy with open lung biopsy can be justified in certain circumstances. Determining the exact nature of pulmonary infiltration at the time of diagnosis of HD is quite important in staging patients and deciding upon the proper type of therapy (see Chapter 50). A growing "coin" lesion excised from one of our asymptomatic patients with CLL proved to be a histioplasmoma.

Therapy of patients with pulmonary infiltration depends upon the type and stage of disease. If HD is otherwise localized and if the pulmonary infiltrates are small and contiguous with hilar or mediastinal disease, "curative" radiotherapy should be directed to the lesion, especially in patients in whom this is found at the time of diagnosis (Chapter 50). However, since the lung is quite sensitive to damage by radiotherapy (Chapter 55), chemotherapy is the treatment of choice in most cases of pulmonary infiltration in leukemia and lymphoma.

Mediastinal structures, commonly lymph nodes, but also the *thymus*, may be infiltrated in patients with ALL,<sup>1,267</sup> CLL,<sup>2</sup> HD,<sup>5</sup> or NHL.<sup>6</sup> An extreme degree of mediastinal enlargement may occur without evident symptoms, but the trachea or superior vena cava may become obstructed.

The *pleura* may be infiltrated with small plaques, or diffusely, in patients with HD,<sup>363</sup> AL,<sup>333</sup> or NHL<sup>6</sup> and we have observed massive, diffuse infiltration of the pleura in one patient with MM. Pain with respiration may be present and pleural effusion usually occurs.

*Serous effusions* of the pleural space, but also of the peritoneal and even pericardial cavities, are common in HD and NHL, complicating the clinical course of 20 to 30% of patients who are not cured.<sup>317</sup> Pleural effusions were found in 60% of autopsied patients with HD in one series and ascitic fluid was present in 44%.<sup>3</sup> Careful cytologic examina-

tion of the effusion revealed diagnostic lymphoma cells in 56% and cells suggestive of lymphoma in another 14%.<sup>317</sup> Thus, it seems likely that infiltration of the serosal surface of either visceral or parietal pleura is the most common cause of effusion. However, autopsy evidence of infiltration was not present in the serosal surface of some patients with effusion.<sup>5,6</sup> Lymphatic obstruction due to enlarged nodes and hypoalbuminemia may contribute to effusion in such patients. Serous effusion also occurs in patients with AL,<sup>1,333</sup> CLL,<sup>2</sup> and CML,<sup>243</sup> but is unusual in those with MM.

When pleural or peritoneal fluid is detected, the fluid should be aspirated for diagnostic purposes unless thrombocytopenia is extremely severe. Infectious exudates or hemorrhage will be present occasionally, even when unsuspected from ancillary signs and symptoms. Chylothorax or chylous ascites may be present, presumably due to obstruction of major lymphatic channels, such as the thoracic duct,<sup>371</sup> by lymphoma<sup>317</sup> or CLL.<sup>300</sup>

Treatment of serous effusions depends, in part, upon their cause. Instillation of anti-tumor drugs into the cavity can be expected to influence serosal or free-growing tumor cells, but will have no effect on lymphatic or venous blockade by tumor and little effect on large, local tumor masses. In general, a trial of systemic chemotherapy is warranted. Intracavitary instillation of drugs should be used for troublesome, recurrent effusions primarily. Instillation of nitrogen mustard (HN2) into the pleural cavity<sup>314,378</sup> remains our treatment of choice because of its high rate of effectiveness after a single dose, relative inexpensiveness, and relative freedom from toxic side effects. Nitrogen mustard is instilled in a dose of 0.4 mg/kg, or less if the marrow is depressed. Some diffusion of HN2 from the cavity is anticipated so that systemic effects may be induced. As much fluid as possible should be removed before instilling HN2 to ensure a high concentration of the drug at the site of tumor. The patient should change position repeatedly and frequently, immediately after instillation of HN2, to aid distribution of the drug throughout the cav-

ity. Pain is rare after intrapleural injection, but is more common after intraperitoneal injection.<sup>314,378</sup> The immediate result may be a rapid increase in fluid, perhaps due to induced inflammation. Indeed, inflammation of the serosa with resultant adhesion between visceral and parietal pleura may be the mode of action of the drug in some circumstances. The possibility of inducing bowel adhesion has made us reluctant to inject HN2 into the peritoneal cavity, but this has been done.<sup>314,378</sup> One dose of HN2 is often sufficient, but the dose may be repeated at intervals of three to four weeks if needed. Intrapleural quinacrine induces a fibrothorax and thereby leads to control of pleural effusion.<sup>248,278</sup> However, the pain and fever resulting from its injection, the need for repeated instillation at frequent intervals, the often delayed response to therapy, and the uninterpretability of future x rays due to the massive pleural thickening caused by quinacrine lead us to prefer HN2. Other agents that have proved helpful in intracavitary treatment of neoplastic effusions include thio-tepa,<sup>372</sup> 5-fluorouracil,<sup>364</sup> and radioactive gold and phosphorus.<sup>239</sup>

### Cardiovascular System

While cardiac infiltration, particularly of the pericardium, can be detected at autopsy in a small but significant number of patients who have died with leukemia, lymphoma, or myeloma,<sup>3,7,9</sup> symptoms and signs are rarely attributable to leukemic infiltration during life. Most instances of arrhythmia, congestive failure, or pericarditis are explainable on the basis of anemia, electrolyte disturbances, infection, hemorrhage,<sup>257</sup> or other secondary factors. However, in certain patients, such factors have not been present and the symptoms and signs have then been attributed to cardiac infiltration because they disappeared following x-ray therapy to the heart, or after chemotherapy. Signs and symptoms of *pericarditis* with pericardial effusion and presumably due to leukemic infiltration of the pericardium have been observed in HD, ALL,<sup>1</sup> AML,

CML,<sup>232</sup> and NHL patients.<sup>6</sup> Pericardial infiltration has been observed during the course of AML when the patient was in bone marrow remission.<sup>228</sup> This is usually a presumptive diagnosis; however, leukemic cells have been demonstrated in pericardial effusions.<sup>328</sup>

Congestive heart failure has been attributed to myocardial infiltration in ALL<sup>279</sup> and CLL.<sup>227</sup> Complete heart block, apparently due to infiltration of the septum, has been reported in association with AML.<sup>250</sup>

*Nonbacterial thrombotic endocarditis* appears to be more frequent in patients with HD, as well as in those with various forms of carcinoma, than in patients dying of non-malignant causes.<sup>349</sup> The nonbacterial endocarditis which is found in patients with "acute eosinophilic leukemia" has been discussed (Chapter 47).

*Vascular obstruction* due to rapidly growing intravascular masses of leukocytes during periods of rapidly increasing leukocyte counts in AL patients is associated with intracerebral leukemia (page 1673). Patients may suffer fatal hepatic and pulmonary destruction by a presumably similar mechanism; any organ can be affected by this process.<sup>316</sup> Infiltration of venous valves has been reported as a cause of venous insufficiency of the legs.<sup>299</sup> However, venous obstruction of clinical significance is more often due to extrinsic masses pressing on large veins than to intrinsic disease of the veins.

The superior vena cava syndrome occasionally is associated with mediastinal lymph node infiltration or with thymic infiltration (page 1678). The inferior vena cava syndrome<sup>229</sup> may be produced by enlarged retroperitoneal nodes in patients with lymphoma. Lymphatic obstruction, hepatic disease, and hypoalbuminemia may contribute to the massive, distal edema associated with obstruction of the vena cava.

Therapy of obstruction of the vena cava should be prompt and vigorous. The site of obstruction can be located accurately by means of venacavography; irradiation directed toward that specific site and concurrent chemotherapy are indicated. Therapy with adrenal corticosteroids is often of benefit,



perhaps by reducing inflammation as well as by shrinking the tumor.

### Breast

The breast occasionally is infiltrated in ALL<sup>1</sup> and AML<sup>354</sup> patients; the infiltration may be diffuse, producing symmetrical breast hypertrophy and even lactation, or it may be nodular.<sup>291a,330</sup> In patients with NHL<sup>8</sup> or HD,<sup>15</sup> nodular breast masses may occur. Localized blastic tumors of the breast have appeared as the first evidence of developing blastic crisis in patients with CML (Chapter 48).

### Liver

Hepatic infiltration and enlargement are commonly associated with AL, CLL, HD, NHL, and with the blastic crisis of CML, but are unusual in MM patients. Infiltration may be diffuse or nodular in nature (Fig.

54-11) in HD, NHL, and in the very rare patient with MM. Leukemic infiltration almost always is diffuse in nature. In AML and CML, leukemic cells are concentrated in perivascular areas, while ALL and CLL more often are associated with marked infiltration of the portal spaces.<sup>3,270</sup> Hepatomegaly may also be the result of hypertrophy of hepatocytes in the leukemias.<sup>259,382</sup>

Evidence of hepatic dysfunction may be absent, particularly in the leukemias, even when hepatomegaly is quite prominent. When troublesome hepatic dysfunction does occur it is usually accompanied by *jaundice*.<sup>308</sup> While jaundice may be due to a variety of complications such as viral hepatitis, bacterial infection, or toxicity from antitumor therapy (Chapter 55), it most often reflects infiltration of the liver by tumor, at least in patients with HD.

Jaundice has been reported in 13% in each of two large series of patients with HD, but its incidence may exceed 50% when analysis



Fig. 54-11 Nodular infiltration of the liver in Hodgkin's disease

is limited to patients dying with HD.<sup>6</sup> The jaundice most often is due to invasion of the liver, less commonly to extrahepatic bile duct obstruction, but it may also reflect hemolytic anemia or be due to other causes.<sup>294</sup>

Obstruction of the common bile duct can occur as the result of infiltration of the duct, compression of the duct by lymph nodes, or even gallbladder infiltration.<sup>374</sup> Obstruction of small biliary radicals may result in a clinical picture similar to that of obstruction of the common duct.<sup>311</sup> Obstruction of the common bile duct accounts for only 3 to 10% of HD patients who are jaundiced.<sup>6</sup>

Portal obstruction by infiltrates in portal spaces with development of esophageal varices has been reported in HD<sup>307,354</sup> and in CLL.<sup>338</sup> Extrahepatic portal vein obstruction has been noted in HD.<sup>308</sup> Hepatic fibrosis with portal hypertension also occurs as a complication of methotrexate therapy (Chapter 55). Portal vein thrombosis in association with thrombocytosis in CML has been reported,<sup>354</sup> but is rare.

Accurate differentiation of the various causes of jaundice is important, but, except for distinguishing jaundice due to hemolysis from jaundice due to liver disease, this is often a difficult task. Clinical features and liver function tests usually are of little help in differentiating liver disease due to tumor invasion from that due to other causes.<sup>6</sup> Alkaline phosphatase and leucine aminopeptidase are often, but not invariably, elevated with tumor invasion. Liver biopsy can be quite helpful and is indicated unless coagulation is abnormal or posthepatic biliary obstruction is suspected.

Systemic chemotherapy is the treatment of choice for hepatic infiltration with hepatic dysfunction. Irradiation, not to exceed 2000 rads, can be given to the entire liver (see Chapter 55) and, if common duct obstruction due to tumor is suspected, irradiation of the area of that structure may be advisable.

### Spleen and Lymph Nodes

The spleen and lymph nodes are less commonly involved in MM and related diseases

(Chapters 52 and 53) than in leukemia (Chapters 47, 48, and 49) or in the lymphomas (Chapters 50 and 51). In most instances it is difficult to attribute specific complications to lymph node or spleen involvement, although, as discussed (page 1663), increased sequestration and/or destruction of normal blood cells can be attributed to splenomegaly in occasional patients.<sup>244</sup>

*Splenic infarction* and, more rarely, *splenic rupture* may occur. Splenic infarction, if it involves the serosal surface of the spleen, may be accompanied by symptoms, as well as signs, suggestive of the primary process. These depend upon whether the inflamed splenic surface infringes upon and irritates the peritoneum, producing left upper quadrant pain, or the serosal surface of the diaphragm, producing pain in the left side of the chest or even in the left shoulder. In either case, auscultation of the left upper quadrant often discloses a rub which is accentuated by deep inspiration, as is pain. Splenic infarction, as judged by the signs and symptoms described above as well as by autopsy,<sup>1,2,3,5,7,8,367a</sup> occurs infrequently in AML or CML and is rare, but may occur, in ALL, CLL, and NHL. It is most unusual in MM.<sup>299</sup>

Rupture of the spleen has been reported as the first manifestation of CML,<sup>315</sup> ALL, or AML<sup>383</sup> and may be observed in patients with CLL,<sup>271</sup> but is a most unusual<sup>290</sup> complication in these disorders or in lymphoma or myeloma,<sup>284,299</sup> despite the frequency of splenic enlargement.

There is no consistent relationship between splenic infarction and splenic rupture. Therapy of the former is limited to simple observation. Symptoms usually disappear within three to five days. If signs of splenic rupture are present (generalized peritoneal irritation with severe pain in the left upper quadrant), immediate surgical exploration with splenectomy is the treatment of choice.

### Gastrointestinal Tract<sup>343</sup>

**ESOPHAGUS.** Symptomatic infiltration of the esophagus is quite rare, but dysphagia due

to infiltration in ALL<sup>226</sup> and dysphagia and obstruction in AML<sup>256</sup> have been reported, as has bronchoesophageal fistula in HD.<sup>328a</sup> Esophageal infiltration has been the site of fatal hemorrhage in ALL.<sup>343</sup>

**STOMACH** As discussed in Chapter 48, peptic ulceration is a common complication in CML as it is in polycythemia vera (Chapter 30), although symptomatic leukemic infiltration of the stomach in CML is most unusual.<sup>241</sup> The frequency of symptomatic infiltrates is difficult to establish since many of the drugs used in therapy produce gastric symptoms. Ulcerated leukemic infiltrates have been observed at autopsy in AML and ALL patients.<sup>343</sup> Massive infiltration (see Fig. 51-6) with retention of gastric contents is observed rarely in patients with acute or chronic leukemia, HD, or NHL.<sup>211,343</sup> Hyperacidity has been reported in children with ALL.<sup>369</sup>

**BOWEL.** Infiltrates of the bowel in acute and chronic leukemia, NHL, and HD are more often found in the small than in the large intestine and are more frequent in the distal ileum than in the proximal small intestine.<sup>319,343</sup> These infiltrates often appear to be portals of entry for infection by intestinal bacteria. Perforation and fistula formation may occur and large areas of gangrenous bowel may be found.<sup>343</sup> Perforation due to rapid lysis of lesions by chemotherapy has been noted in HD.<sup>6</sup> Nodular lesions or lesions that become polypoid occur in all forms of leukemia<sup>313</sup> and have been associated with intussusception and intestinal obstruction.<sup>274,297,298,362</sup> Massive, diffuse infiltration of large segments of the stomach or bowel is seen occasionally in CLL (Fig. 54-12)<sup>313</sup> and has been noted during bone marrow remission in acute leukemia.<sup>255</sup> We have observed a patient with NHL in whom virtually the entire bowel was infiltrated. Diffuse or multifocal lesions of NHL, and less commonly of HD, in the proximal small intestine may produce severe degrees of malabsorption.<sup>337,344</sup> This syndrome seems to be much more common in Arabs, Sephardic Jews,

Mexicans, and the South African Cape colored than in the general population of the United States.<sup>337,344</sup> It may be more common when NHL is limited to the bowel than as a secondary feature of NHL.<sup>337,344</sup>

Primary NHL of the bowel is discussed in Chapter 51. HD apparently originating in and limited to the bowel may occur.<sup>6</sup>

Lesions located in the sigmoid colon and rectum are frequently associated with symptoms of rectal pain and mucosanguineous diarrhea.<sup>343</sup> As noted elsewhere (page 1656), rectal infections are serious complications in ocrotropic patients.

So-called pseudo-acute abdomen has been reported in ALL and AML<sup>343</sup> as well as in HD.<sup>215</sup> Signs and symptoms suggesting intestinal obstruction with perforation and peritonitis were present, but at operation or autopsy no perforation could be demonstrated.

### Renal Complications

The renal complications seen in multiple myeloma and related diseases that are primarily associated with overproduction of light chains are discussed in Chapter 52. Symptomatic complications or significant impairment of renal function is more often due to pyelonephritis (page 1656), hemorrhage (page 1662), hyperuricemia (page 1689), hypercalcemia (page 1692), drug toxicity (Chapter 55), or ureteral obstruction by tumor masses (page 1684) than to renal infiltration.<sup>345</sup> Nevertheless, infiltration of kidneys producing renal failure may occur in leukemia and lymphoma.<sup>5,8</sup> In a series of 178 patients with AML and ALL, four developed otherwise unexplained azotemia in association with markedly enlarged, infiltrated kidneys.<sup>260</sup> However, normal renal function has been noted in other patients with AL in whom marked renal enlargement and infiltration were present.<sup>260</sup> Functional impairment also has been reported in a few patients with CLL, HD, or NHL when there was diffuse or nodular infiltration.<sup>346,356</sup> The nephrotic syndrome,<sup>356</sup> as well as azotemia,<sup>346,356</sup> may be associated with such infiltration, but fewer than 10% of patients with HD or NHL in-



Fig 54-12 Enormously enlarged stomach with mucosa thickened and thrown into huge convolutions in a patient with chronic lymphocytic leukemia (WBC  $86 \times 10^9/l$  lymphocytes 96%) Some of the convolutions were as much as 2 cm thick and 2 cm high

volving the kidneys at autopsy exhibited azotemia<sup>346</sup> from any cause.

The diagnosis of impairment of renal function due to renal invasion by leukemia or lymphoma is one of exclusion. The urinary findings are nonspecific, consisting primarily of albuminuria, rarely of hematuria and pyuria.<sup>346,356</sup> Since most infiltrates are not associated with impairment, biopsy proof of infiltration does not indicate that impairment is due to infiltration. In the absence of hyperuricemia, hypercalcemia, positive urine culture, or recent therapy, particularly x-ray therapy to renal areas, and in the presence

of roentgenographic evidence of renal enlargement or tumors one assumes that renal invasion is the correct diagnosis. Intravenous pyelography poses some hazard to patients with multiple myeloma in that anuria and even death have been reported to follow the procedure<sup>305a</sup> (Chapter 52). While it has been suggested that this does not occur unless the patient is dehydrated<sup>305a</sup> we have observed sudden onset of anuria following an IVP in a patient with MM in whom no dehydration was induced. "Test doses" of the dye used in pyelography have no value in predicting subsequent fatal reactions.<sup>257a</sup>

Therapy of renal infiltration is best accomplished by systemic chemotherapy, but if this proves of no avail x-ray therapy may be directed to the kidney.<sup>246, 256</sup> However, dosage should probably not exceed 1000 rads as the kidney is easily damaged by irradiation (Chapter 55).

Renal hypertrophy without leukemic infiltration and also without functional impairment is fairly frequent in AL and is of unknown cause.<sup>264</sup>

The *nephrotic syndrome*, in the absence of renal infiltration and without other demonstrable cause, may develop in association with HD.<sup>246, 256a, 277, 283, 288</sup> There are at least 30 case reports dealing with such occurrences. The disappearance of the syndrome with therapy of HD suggests a causative relationship,<sup>246</sup> and a similar observation has been made in Burkitt's lymphoma.<sup>283</sup> However, how HD, in some cases localized to distant structures such as the mediastinum,<sup>277</sup> can produce a nephrotic syndrome remains to be clarified. It has been suggested that this is immune complex renal disease, representing deposition of tumor specific antigen-antibody complexes;<sup>256a, 293;</sup> such deposits have been demonstrated in glomeruli of mice with spontaneously occurring leukemia.<sup>237b</sup> The nephrotic syndrome also has been associated with CLL in the absence of renal infiltration, but in these cases there was excessive production of light chains.<sup>236</sup> Therefore, the nephrotic syndrome may have been produced by the same mechanism as is suggested in MM (Chapter 52). A patient with RCSa associated with anasarca and hypoproteinemia, but without proteinuria, which were reversible with therapy and was thought to be due to infiltration has been reported.<sup>295</sup>

### Genitourinary Tract<sup>310</sup>

The testes, penis, bladder, ureters, prostate gland, uterus, and ovaries may be infiltrated by tumor. Infiltration of these organs is most common in AL. Testicular infiltration, unilateral or bilateral, occurs in a small percentage of patients with ALL; the testis is one of the sites in which localized leukemia

develops in patients who otherwise remain in complete remission.<sup>1</sup> The testes may be involved in lymphoma. A testicular mass may be the presenting complaint of such patients, but generalized disease usually is found.<sup>276</sup> Involvement is most often unilateral, but subsequent invasion of the opposite testis is fairly common.<sup>276</sup> In one of our patients, a 10 year old boy with ALL, removal of one involved testis was followed by infiltration of the other within six months, although the patient remained in complete remission otherwise throughout this period and for two years following irradiation therapy to the second testis.

*Priapism* is observed in an occasional patient with CML or AL and persistent clitoral engorgement has also been reported in patients with CML.<sup>331</sup> Priapism is thought to be due to infiltration of the corpora cavernosa and may regress following systemic chemotherapy or local irradiation.

*Prostatic infiltration* producing obstructive symptoms has been reported in AML and CLL patients; it developed during bone marrow remission in one patient with AML.<sup>322</sup> The *bladder* may be infiltrated in any form of leukemia or lymphoma.<sup>268, 275</sup> Though rare,<sup>6, 268</sup> more than 40 cases of NHL apparently localized in the bladder have been reported.<sup>375</sup> If symptoms are produced by bladder lesions, they consist of those associated with any tumor of the bladder, such as hematuria, dysuria, and frequent urination.

Ureteral infiltration, severe enough to produce obstructive uropathy, has been noted in NHL, HD, and CLL.<sup>268</sup> Ureteral obstruction is likely to be due to encroachment upon ureters by enlarged retroperitoneal nodes.<sup>8</sup>

Invasion of uterus and ovaries may occur, but rarely produces symptoms.<sup>3, 381</sup> However, fatal ovarian hemorrhage has been reported.<sup>159</sup>

### Endocrine Glands

Infiltration of the *thyroid gland* may lead to goiter in patients with HD,<sup>8</sup> as may hemorrhage into this gland in those with AML.<sup>351</sup> Although the basal metabolic rate

is often increased in leukemia and lymphoma, thyroid dysfunction is unusual (page 1688). Lymphomatous tissue may take up radioactive iodine at a greater rate than does normal, nonthyroidal tissue, leading to low thyroid uptake without hypothyroidism.<sup>310</sup>

The adrenal gland rarely is affected significantly by infiltration in leukemia and lymphoma. Small infiltrates have been demonstrated at autopsy in a few patients,<sup>3,7</sup> but studies of adrenal hormone secretion disclose normal or slightly increased secretion, presumably as a nonspecific response to stress.<sup>339,350</sup> Hemorrhage into the adrenals has been considered a cause of death in a patient with CML.<sup>304</sup>

The endocrine function of the pancreas usually is normal. Hypoglycemia does not appear to be a complication of hematologic neoplasms although it may occur artifactually in leukemia when the blood leukocyte count is markedly elevated.<sup>397,417</sup> Glucose is utilized by leukocytes in blood samples after they have been drawn unless a larger than normal amount of sodium fluoride is added to inhibit glycolysis. Onset of diabetes has been noted coincidentally with onset of leukemia,<sup>415</sup> but most diabetic problems are associated with adrenal corticosteroid therapy (Chapter 55). Diabetes insipidus has been produced by infiltration of the posterior pituitary in AL<sup>321</sup> and CML patients.<sup>304</sup> Forty-six percent of 52 patients with AL were found to have peripituitary gland infiltration, but in none was there evidence of clinically significant dysfunction.<sup>314a</sup> Inappropriate antidiuretic hormone secretion has been reported in at least five patients with HD; this disappeared with chemotherapy of HD. The source of the hormone, whether from pituitary gland or from tumor elsewhere, has not been established.<sup>240</sup> Ovarian and testicular involvement were discussed in the preceding section.

### Musculoskeletal System

Destruction of bone with severe pain is much more commonly associated with MM (Chapter 52) than with other hematologic

neoplasms. The reason for this tumor's bone-destroying propensity has not been clarified. Bone pain with roentgenographic abnormalities of bone is common in AL, occurs with some frequency in NHL and HD, and is rare in CLL and in CML except during blastic crises.<sup>329,368</sup>

Bone pain occurs in approximately 50% of patients with AL<sup>368</sup> and is somewhat more common in ALL than AML patients of any age. In children with ALL the pain is most often in the long bones, while in adults with AML it more often is in ribs and vertebrae.<sup>368</sup> Diffuse osteoporosis, associated with enlargement of haversian and Volkmann's canals by leukemic infiltration, probably accounts for some instances of bone pain. Radiolucent metaphyseal bands are present in the long bones of almost all children with AL (Fig. 54-13). These are not due to leukemic infiltration and do not correlate with bone pain but represent arrest of bone growth during periods of active disease. The number of relapses and remissions that children have experienced is often mirrored by a like num-



Fig. 54-13. Metaphyseal lines in the tibia of a child with ALL.



Fig. 54-14 Roentgenograms of the humerus of an 11 year old girl with acute lymphoblastic leukemia. Note the irregular distortion of the architectural pattern and the multiple defects within the medullary portion of the bone: the decalcification of the cortex on the mesial aspect of the shaft and the periosteal proliferation.

ber of metaphyseal bands. Osteolytic lesions, resulting from leukemic infiltrates, or less often from bone infarcts, are common and may be associated with pain.<sup>334</sup> Cortical destruction (Fig. 54-14) with or without periosteal elevation appears to be the most common cause of pain in the extremities in ALL patients. Necrosis of bone from bone infarcts produces pain before it is demonstrable roentgenographically. Pain from bone destruction may be the primary symptom of blastic crisis in CML.<sup>342</sup>

Bone pain and bone lesions occur in perhaps 10% of patients with HD or NHL (Fig. 54-15). They may represent invasion of bone from lesions in bone marrow or direct extension to bone from disease in lymph nodes. Osteolytic areas of bone are more usual (Fig. 54-16), but an osteoblastic x-ray picture may also be observed.

Pathologic fractures are unusual, except in patients with MM (Chapter 52), but have been reported in AL, CLL, NHL, and HD.<sup>312,368</sup>

Metastatic acropachy with extreme enlargement of the terminal phalanges due to infiltration has been reported in a patient with CLL.<sup>237</sup>

*Joint pain* occurs in 10 to 20% of patients with AL, but is uncommon in those with CML, CLL, or lymphoma.<sup>368</sup> Arthritis as



Fig. 54-15 Destructive process involving the proximal end of the right radius with marked periosteal reaction over it, in a patient with lymphosarcoma (lymphoblastic lymphoma).

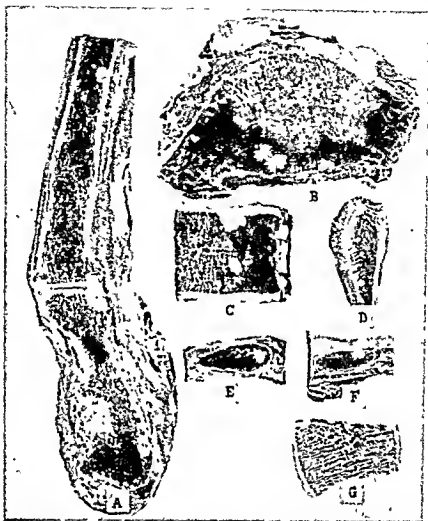


Fig 54-16 Lymphogranulomatous lesions in the marrow in a patient with Hodgkin's disease. A, Manubrium and half of the sternum. B, Transection through the fourth lumbar vertebral body and C, mid-dorsal vertebral body. D, Right iliac crest, and E and F the right third and second ribs. G, The only bone without a lesion, the left first metatarsal (Steiner,<sup>364</sup> courtesy of Archives of Pathology.)

well as arthralgia may be present. The arthritis tends to be symmetrical and migratory and most commonly involves the hands, feet, elbows, ankles, and knees. The cause of the arthritis is not entirely clear; the condition may be due to infiltration of the metaphyseal periosteum subjacent to the joint capsule with associated inflammation and effusion.<sup>368</sup> Synovial infiltration is quite rare.<sup>368</sup>

Muscle infiltration by leukemia or lymphoma is most unusual. Polymyositis of unknown cause has been associated with AML<sup>254</sup> and complaints of muscle ache are not uncommon in patients with AL.

## Skin

Disease, apparently primary in the skin, such as occurs with mycosis fungoides and the Sézary syndrome was discussed in Chapter 51.

Infiltration of the skin may occur in any of the leukemias or lymphomas, but is quite rare in MM. Leukemic infiltration of the skin was noted in 13% of our patients with AML, but in only 1% of those with ALL.<sup>1</sup> Although it has been suggested that skin infiltration is most frequent with monocytic leukemia,<sup>235</sup> this was not the case in our series.<sup>1</sup> Skin



infiltration may occur in patients with CLL and is least common in those with CML.<sup>235</sup> It complicates the course of approximately 5% of patients with HD or NHL.<sup>6,8</sup>

In AML, skin infiltrations are often diffuse, slightly raised, and dark reddish-brown to purple, although nodular lesions may occur (Fig. 54-17). In the other neoplastic diseases of the hematopoietic system, discrete intra- or subcutaneous nodules and plaques are more common. They may have the same color as the skin or appear erythematous. Ulceration of the overlying skin may occur (Fig. 54-18). The nodules may be painful, but more often are not, and diffuse infiltration may be associated with pruritus. Pruritus, as a symptom in HD, usually does not reflect skin infiltration (Chapter 50). On occasion, localized edema may surround infiltrates.

In the differential diagnosis of skin infiltrates, any other type of skin tumor as well as a variety of papular eruptions must be considered. If doubt exists regarding the na-

ture of the lesion, a small punch biopsy is advisable.

Systemic chemotherapy is indicated in most patients since skin infiltration is usually associated with widespread disease. However, local irradiation or even local application of chemotherapeutic agents (see Chapter 51) is effective. Treatment of noninfiltrative pruritus in HD and NHL should be directed at the primary disease, but some symptomatic relief has been reported with levomepromazine therapy.<sup>231</sup>

The term *leukemid* has been applied to the myriad of noninfiltrative forms of skin lesions associated with leukemia.<sup>1,235</sup> Severe skin diseases have been associated with leukemia and lymphoma and the association is unexplained. Examples include ichthyosis with severe anhydrosis,<sup>252</sup> erythema nodosum,<sup>311</sup> exfoliative dermatitis,<sup>222,325</sup> and pyoderma gangrenosum.<sup>310a</sup>

## Complications Due to Metabolic Imbalance

In any serious disease in which complex forms of therapy are employed, a wide variety of disturbances in fluid and electrolyte balance are to be anticipated. In this section we are concerned primarily with certain specific disturbances, such as hyperuricemia (page 1689) and hypercalcemia (page 1692), produced by the presence of hematopoietic neoplasms.

A negative nitrogen balance and weight loss commonly accompany all forms of leukemia, lymphoma, and myeloma. However, the degree of weight loss is quite variable and often is not a prominent feature in the leukemias, while, in contrast, in the terminal phases of HD many patients are cachectic. Anorexia, either due to disease or as a common side effect of many drugs (Chapter 55), is certainly a primary factor in inducing weight loss in many patients with leukemia, lymphoma, or myeloma. In extreme cases, intravenous hyperalimentation has been employed.<sup>423a</sup> The basal metabolic rate is abnormally high in many patients, even in the absence of fever.<sup>420</sup> Other studies of thyroid function usually give results that are within

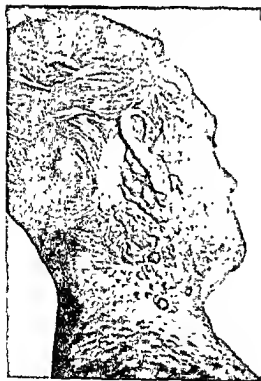
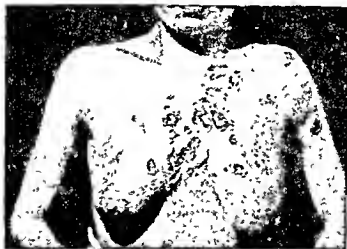


Fig. 54-17. Myeloid leukemia of the skin (Ketron and Gay<sup>271a</sup> courtesy of the authors and Archives of Dermatology)



A



B

Fig. 54-18. Nodular (A) and ulcerative (B) skin lesions in Hodgkin's disease (Jackson and Parker,<sup>285a</sup> courtesy of the authors and New England Journal of Medicine)

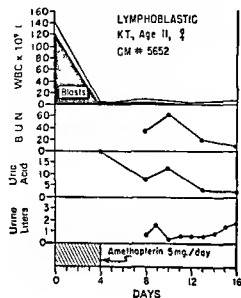
normal limits; the increased  $O_2$  utilization may reflect the growth requirements of the tumor cells. It seems possible that the increased  $O_2$  demands contribute to fatigue as well as to weight loss.

### Hyperuricemia

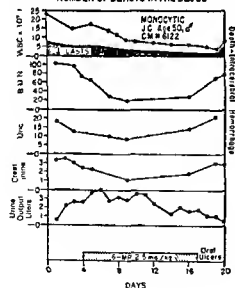
Hyperuricemia is a common manifestation that probably reflects an increased rate of purine metabolism due to rapid turnover of

the tumor cells. However, other factors, such as decreased plasma uricolytic,<sup>393</sup> may contribute to hyperuricemia. Abnormally high serum levels are common in untreated patients with AL or CML<sup>395,404,405</sup> as they are in those with polycythemia vera (Chapter 30) or idiopathic myelofibrosis (Chapter 57). Hyperuricemia is less common in untreated CLL, HD, NHL, or MM.<sup>395,404,416</sup> As one would expect, increased excretion of urate can be demonstrated in a greater proportion

# HYPERURICEMIA AND UREMIA ACCOMPANYING A RAPID DECREASE IN NUMBER OF BLASTS IN THE BLOOD WITH THERAPY



# HYPERURICEMIA AND UREMIA WITH A RELATIVELY STABLE NUMBER OF BLASTS IN THE BLOOD



of patients than can hyperuricemia.<sup>416</sup> Antitumor therapy leads to a sudden acceleration of purine breakdown and uric acid production. Hyperuricemic episodes in AL are shown in Figure 54-19.

# HYPERURICEMIA AND UREMIA WITH INCREASING NUMBERS OF BLASTS IN THE BLOOD

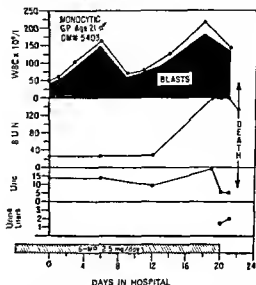


Fig 54-19. Hyperuricemia in acute leukemia (From Boggs et al.,<sup>1</sup> courtesy of the authors and Williams & Wilkins Company)

Gouty arthritis may occur but is not a particularly common complication. Gouty tophi are rarely, if ever, seen.<sup>424</sup> This may simply reflect the relatively short duration of hyperuricemia in leukemia and lymphoma as compared to that in idiopathic gout.

Urate nephropathy is a serious, but usually avoidable, complication of hyperuricemia that may develop when serum uric acid levels exceed 10 mg/dl. Any degree of renal failure in the presence of hyperuricemia should be considered presumptive evidence for urate nephropathy. The physicochemical properties of uric acid are important in the pathogenesis of urate nephropathy.<sup>260</sup> Ionized urate is much more soluble than the unionized form. The pK of uric acid is 5.4 so that, at normal blood pH, virtually all uric acid is ionized, but, at urine pH of less than 5.4, less than 50% is ionized. At normal blood pH, uric acid may remain ionized, even in supersaturated solution, explaining why serum urate concentrations in excess of saturation may be present without intravascular

crystal formation. However, the hyperosmolar, maximally acid distal tubules and proximal collecting ducts of the kidney provide an ideal milieu for precipitation of urate crystals. Urate nephropathy appears to be the direct result of precipitation of crystals in the distal tubules and collecting ducts with consequent mechanical obstruction. It may develop in untreated patients (Fig. 54-19) and may even be the presenting feature in AL,<sup>414</sup> but the degree of urate load required to induce this complication usually is associated with cytotoxic therapy. In this setting we have observed serum uric acid levels as high as 88 mg/dl.

Oliguria or, in severe cases, anuria develops and is accompanied by a rising BUN and all the accompanying features of acutely developing uremia. It has been suggested that extreme hyperuricemia in and of itself may produce anorexia, nausea and vomiting, lethargy and weakness since these symptoms have been observed to begin during uric acid nephropathy and before severe uremia had developed.<sup>421</sup>

Uric acid calculi may be formed and typical renal colic and, in some patients, ureteral obstruction may ensue.<sup>253</sup>

*Therapy of urate nephropathy rarely is necessary if proper attention is given to prevention of the complication. The use of allopurinol to reduce uric acid production prior to or concurrent with the initiation of cytotoxic therapy is a critical factor in preventing urate nephropathy.*<sup>395,405</sup> Allopurinol (4-hydroxyxypurazole[3,4-d]pyrimidine) is an isomer of hypoxanthine which inhibits xanthine oxidase. This enzyme is responsible for oxidation of hypoxanthine, converting it to xanthine and then to uric acid. Administration of 300 to 800 mg of allopurinol per day results in a significant reduction of serum and urinary uric acid and an increase in the oxypurines, hypoxanthine and xanthine, in urine.<sup>395,405</sup> For reasons yet to be determined, oxypurine excretion is not increased commensurate with the decrease in uric acid excretion, a factor which may be the primary reason for the usefulness of allopurinol in preventing nephropathy since the solubility

of oxypurines in the urine is similar to that of uric acid.<sup>405</sup> Indeed, on rare occasion, xanthine nephropathy may be associated with allopurinol therapy.<sup>399</sup> In addition to giving allopurinol, proper hydration, designed to produce increased urine flow and reduced osmolality of urine in the collecting ducts, is important. Alkalinization of urine by administration of sodium bicarbonate or acetazolamide is potentially useful, but is probably not necessary in prophylactic regimens.

If hyperuricemia is present at the time of diagnosis, it is wise to lower the serum uric acid by means of allopurinol and hyperhydration before giving cytotoxic drugs. This usually requires no more than two to three days and a therapeutic delay of this duration is rarely critical. The concurrent initiation of allopurinol with cytotoxic therapy in hyperuricemic patients may fail to prevent urate nephropathy.<sup>407</sup>

If this course is followed, urate nephropathy should be seen in only the occasional untreated patient whose tumor cell turnover rate is high enough to produce de novo nephropathy. However, oxypurine nephropathy should be anticipated occasionally.

The type of treatment for urate nephropathy depends, in part, upon whether the condition has progressed to the anuric stage. If oliguria is present, prompt but cautious hyperhydration is indicated, but excessive fluid should not be given. With oliguria, alkalinization of the urine with bicarbonate (100 mEq m<sup>2</sup>/24 hr) should be attempted.<sup>260</sup> Uricosuric agents should be avoided since reduced rather than increased<sup>122</sup> urinary uric acid is the goal. Allopurinol therapy, if not in use, should be instituted immediately. No form of antitumor therapy should be given during the period of urate nephropathy. In the anuric patient, as in any type of acute renal shutdown, the measures designed to carefully monitor and treat fluid and electrolyte balance are crucial. Diuresis induction with mannitol may be attempted, but if urine flow does not begin, repeated trials are not advisable.<sup>253</sup> Hemodialysis or peritoneal dialysis may be employed,<sup>389</sup> but usually is unnecessary. Spontaneous diuresis usually develops within a

week except in those who die from concurrent infection or hemorrhage.

Hypouricemia has been reported in HD and was apparently the result of a tubular defect resulting in excessive secretion of uric acid.<sup>392</sup>

### Hypercalcemia

Symptomatic hypercalcemia, common in MM (Chapter 52), may occur with any form of leukemia or lymphoma, but is not common.<sup>412</sup> Among these conditions it is probably most often seen in ALL,<sup>423</sup> and perhaps is least common in HD, CML, or CLL.<sup>401, 422</sup> Destructive bone lesions have been present in most patients and have been presumed to be the cause of the hypercalcemia,<sup>401</sup> but parathyroid infiltration has also been reported as a cause in AL.<sup>415</sup> Increased serum levels of parathormone were found in a child with AL in whom the parathyroids appeared normal at autopsy, suggesting that leukemic cells might be the source of the excess hormone.<sup>413</sup> Neither bone lesions nor excessive parathormone could be found in a patient with RCSa and hypercalcemia, but an extract of the tumor increased the serum calcium level when injected into mice.<sup>420a</sup> Although hypercalcemia has been a presenting feature of disease in ALL,<sup>406</sup> in most patients it has accompanied terminal or pre-terminal disease.<sup>401, 423</sup>

The array of symptoms and signs associated with hypercalcemia is almost limitless, but these usually can be divided into neurologic manifestations such as drowsiness, confusion, coma, and muscular weakness; gastrointestinal symptoms such as anorexia, nausea, and vomiting; renal disease with hyposthenuria and proteinuria; cardiac arrhythmias and other associated electrolyte disturbances such as hypokalemia.<sup>412</sup> Therapy should be directed toward alleviating the primary disease as well as toward lowering serum calcium per se. As discussed in Chapter 52, this includes various combinations of diuresis, phosphate loading, and therapy with adrenal corticosteroids and mithromycin.<sup>401</sup>

Unexplained hypocalcemia has been reported in ALL.<sup>422a</sup>

### Serum Proteins

*Albumin* levels in serum often are decreased in all of these diseases and tend to be decreased in direct proportion to the severity of the disease.<sup>394, 396</sup> (Fig. 54-3). In lymphoma, hypoalbuminemia usually reflects reduced rates of albumin synthesis or a shift of serum albumin into protein-rich effusions.<sup>426</sup> The cause of the reduced synthesis is unexplained.<sup>426</sup> Malnutrition, malabsorption, liver disease, diversion of nitrogenous precursors for tumor growth, and albumin loss in urine and from the bowel (page 1682) may contribute to the hypoalbuminemia.

Hypoalbuminemia may aggravate edema which results from lymphatic blockade or venous blockage (page 1679) and contributes to the accumulation of serous effusion (page 1678).

The *globulins* are often changed in concentration.<sup>394, 396</sup> Alpha-2 globulin is likely to be markedly elevated in HD (Chapter 50, Fig. 54-3). Nonspecific changes often are present in alpha-1, alpha-2, and beta globulins and the former two are frequently elevated when fever is present.<sup>32</sup> Changes in gamma globulins have been discussed (page 1654).

Cryoglobulinemia, of symptomatic degree, may complicate the course of AL and NHL as well as that of MM (Chapter 52). Cryofibrinogenemia has been reported in AML.<sup>398</sup>

Paraproteins may be observed occasionally in diseases other than myeloma, as discussed in Chapter 53. The hyperviscosity syndrome, which is associated with high serum paraprotein levels, has been discussed (Chapter 53).

*Other types of metabolic defects* abound, but their significance is unclear. Some examples will be given, but a complete survey is not intended. Urinary glycoproteins are increased in all forms of leukemia.<sup>425</sup>  $\beta$ -Amino-isobutyric acid, a metabolite not ordinarily found in urine, is present in some patients with any form of leukemia, but not in those with HD

or MM.<sup>403</sup> An increased excretion of 4-amino-5-imidazolecarboxamide was noted in patients with acute leukemia, but not in those with other hematologic neoplasms.<sup>408</sup> Levels of certain trace metals have been found to be altered in some patients; eg, low plasma magnesium was reported in a patient with HD, but high magnesium levels were found in the red cells of patients with acute leukemia. Nickel levels were increased or decreased in plasma and red cells in various patients; elevated plasma copper was found in some patients with HD, NHL, or leukemia, but chromium levels were consistently normal.<sup>402</sup> Plasma unesterified fatty acid concentration often is abnormally high and cholesterol is low in persons with hematologic malignant disease, as it is in persons with other forms of cancer.<sup>391,411</sup> Creatinine excretion was found to be decreased in all forms of leukemia, but creatine was increased in the urine.<sup>338</sup>

In AML patients,<sup>415</sup> false positive reactions to serologic tests for syphilis have been reported.

*Amyloidosis* is a common complication of MM (see Chapter 52) and, indeed, there is evidence to suggest that so-called "primary amyloidosis" may be a form of plasma cell dyscrasia.<sup>390</sup> This complication rarely is observed in leukemia or lymphoma, although it has been reported in lymphoma.<sup>291</sup>

## Pregnancy<sup>430</sup>

Pregnancy cannot be considered a "complication" of the diseases under discussion. Yet, a large body of literature is concerned with pregnancy developing during the course of these diseases and discusses the diagnosis of these diseases during pregnancy, at the time of delivery, or during the postpartum period. The frequency with which pregnancy has been reported to occur in women with various hematologic malignant conditions would appear to reflect the duration of survival associated with the tumor, the relative frequency of the tumor, and the age distribution of women with the tumor. Pregnancy

has been reported more often in women with HD or CML than in those with the acute leukemias.<sup>430</sup> Less frequent is pregnancy in women with NHL<sup>439</sup> and pregnancy is quite unusual in those having CLL<sup>432</sup> or MM,<sup>434</sup> diseases which are relatively rare in patients in the child-bearing age.

Three essential questions need to be approached in discussing the interrelation of pregnancy and hematologic malignant conditions: (1) What effect, if any, does pregnancy exert upon the tumor? (2) Does the tumor adversely affect pregnancy? (3) How does the interrelation of pregnancy and tumor influence therapy of the tumor?

The suggestion was raised that patients with AL or HD who become pregnant are more likely to improve spontaneously than nonpregnant patients.<sup>433</sup> This suggestion is not well documented and the opposite impression has been aired.<sup>436,451,456</sup> Pregnancy did not influence survival time of mice transplanted with a myeloid leukemia.<sup>444</sup> In general, it would appear that pregnancy has neither a salutary nor an adverse effect upon HD<sup>431,457</sup> or leukemia.<sup>430,441,443</sup>

The chance of delivering a full-term, healthy child is certainly reduced if AL is diagnosed during pregnancy.<sup>430</sup> This is less true in CML.<sup>430</sup> The possibly increased hazard of a child born of a leukemic mother developing leukemia has been discussed (Chapter 46). Transient pancytopenia has been recorded in a child born of a mother with untreated AML.<sup>442</sup> Maternal death from hemorrhage or infection at or shortly after delivery is not uncommon.<sup>430</sup>

The outcome of pregnancy occurring during HD and AL in remission or during the course of CML seems to depend primarily upon the type of therapy employed during pregnancy. Some antitumor drugs, such as methotrexate, are so efficient in inducing abortion that they have been used for this purpose.<sup>460</sup> Others, such as busulfan, used in relatively small doses in CML, have been employed during conception.<sup>462</sup> Normal infants have been delivered of mothers treated during the first trimester with 6-MP<sup>441,453</sup>

for acute leukemia; busulfan<sup>462</sup> and demecolcine<sup>447</sup> for CML; vinblastine,<sup>429,446</sup> nitrogen mustard,<sup>437</sup> and cyclophosphamide<sup>446</sup> for HD. However, severe congenital defects have been present in viable infants born of mothers treated for CML with busulfan and 6-MP<sup>458</sup> and for HD with alkylating agents.<sup>445,455</sup> In general, all antitumor therapy, with the possible exception of hormonal therapy, should be considered potentially mutagenic<sup>434, 438, 449, 450, 461</sup> and this consideration should influence the physician's advice and management of all pregnant patients with hematologic malignant disease.

Thus, a general statement can be made to the effect that the hematologic neoplasm adversely affects pregnancy, but does not clearly dictate its interruption; pregnancy does not clearly influence the course of the neoplasm in any fashion; but therapy of the neoplasm is complicated by the presence of pregnancy. These considerations lead to certain practical recommendations, as outlined below.

If NHL or CML is diagnosed during pregnancy, therapy should be withheld in most of these patients since cure is not anticipated and there is no evidence that a delay in therapy will reduce survival unless unusual complications are present (Chapters 49 and 51). In the rare circumstance of pregnancy during CLL or MM, the same considerations may be applied. If AL is diagnosed, the average survival of untreated patients does not exceed three months (Chapter 47). Thus, antitumor therapy should be undertaken unless pregnancy is very near to termination. Abortion will often follow antileukemic therapy undertaken during the first or even the second trimester.<sup>452</sup> Perhaps the most difficult decision concerns the patient in whom a diagnosis of HD is made during pregnancy.<sup>6</sup>

The first situation that can be posed is that of the patient in whom HD has been diagnosed, clinical staging has been completed (Chapter 50), and pregnancy is discovered before therapy has begun. If the disease is limited to areas above the diaphragm and is stage I or II-A, radiotherapy to involved nodes only might be given as a temporizing measure.<sup>6</sup> However, if more extensive disease

is present, requiring immediate extensive irradiation or chemotherapy in order to enhance the chances of cure, abortion should be recommended. Radiation therapy to the groin or even to the entire mediastinum probably entails appreciable risk to the fetus from radiation scatter, even when careful collimation of beams is attempted.<sup>435</sup> When HD is discovered during pregnancy, therapeutic abortion or cesarean delivery, if the fetus is potentially viable, may be advisable in order to permit clinical staging and definitive treatment.<sup>6</sup> In all cases, it must be recognized that if the mother chooses not to be aborted, then the physician must still design the best form of treatment that is consistent with the delivery of a completely healthy infant.

If pregnancy coincides with relapse of HD when cure of disease is no longer likely, further therapy often can be delayed until after delivery.

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# *Principles of Therapy and Effects of Specific Drugs Used in Therapy of Neoplastic Diseases of the Hematopoietic System*

Approach to the Patient with a Proven Hematologic Neoplasm

Agents Useful in the Treatment of Hematologic Neoplasms

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Vinblastine

Vincristine

Other Stathmokinetic Agents

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Daunomycin

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Other Antibiotics

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Procarbazine

Nitrosoureas

Other Agents

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Bone Marrow Transplantation

Immunotherapy

Other Forms of Therapy

Development of New Agents

THE principles underlying the use of radiotherapy, chemotherapy, or other modalities of treatment are the same for all types of hematologic malignant diseases but details concerning their use differ, as discussed in Chapters 47 to 53. In this chapter the general principles of therapy, the mechanism of action of each useful form of therapy, and the associated toxicity are considered. The treatment of complications was discussed in Chapter 54.

## **Approach to the Patient with a Proven Hematologic Neoplasm**

It is important to develop a specific plan of therapy for each patient, taking into account the nature of the tumor, its natural



course if left untreated, whether cure is feasible, and also such variables as age, psychologic aspects, economic status, coexisting diseases, and the presence or absence of complications associated with the tumor.

The importance of distinguishing between *therapy with curative intent* and *palliative therapy* for each disease has been discussed previously, but deserves reemphasis. Cure is a practical therapeutic objective in most patients with Hodgkin's disease (Chapter 50) and in certain patients with non-Hodgkin's lymphoma (NHL) (Chapter 51), even if the goal is not attained. With presently available forms of therapy, cure is not a reasonable possibility in the *chronic leukemias* (Chapters 48 and 49), in acute myeloblastic leukemia (Chapter 47), in multiple myeloma (Chapter 52) and in other protein-secreting tumors (Chapter 53), or in most patients with NHL. The dilemma of whether acute lymphoblastic leukemia should or should not be approached routinely with intent to cure has been discussed (Chapter 47). Continuing attempts to cure patients with these diseases by experimental forms of therapy are exceedingly important, but until evidence is forthcoming that cure is possible, palliative therapy remains the treatment of choice for patients who have not volunteered for studies in research institutions.

The goal of palliative therapy is simple to state but frequently difficult to achieve. This is to keep the patient's physical and emotional health as optimal as possible for as long as possible. If cure is not possible by presently available means, if the patient is asymptomatic, and if the known natural course of most patients with the disease suggests that the patient is likely to remain asymptomatic for some time, it is usually best to give no antitumor therapy. Watchful waiting is particularly useful in the management of many patients with chronic lymphocytic leukemia. In other circumstances, therapy may be advisable in an asymptomatic patient because the natural course of the disease is such that the asymptomatic period will be terminated shortly unless therapy is given. For example, the patient with CML who is beginning to

relapse from busulfan-induced remission is likely to benefit from re-treatment with busulfan and nothing is gained by waiting for florid relapse to develop.

If cure is the goal, and if iatrogenic complications must be induced to attain that goal, even life-threatening toxicity may be acceptable. Conversely, if palliation is the goal, attention to the relative toxicity of various therapeutic regimens becomes especially important, and the least potentially toxic therapeutic agent should be chosen.

The physician also should consider the relative expense of various forms of palliative therapy because he should attempt to avoid superimposing "medical indigency" in an already troubled family. This entails less the actual cost of drugs than it does the frequency of hospitalization, office visits, and laboratory and radiologic procedures required by various regimens.

The age of the patient and the presence of other diseases must also be considered. In the opinion of some, radical radiotherapy either should not be carried out or should at least be modified in the elderly.<sup>96</sup> The reduced remission rate in elderly patients with AML appears to reflect increased drug toxicity primarily (Chapter 47), which suggests that less aggressive therapy should be employed in the aged than in younger patients.

The physician must be willing to deal with and care for all needs of the patient, most particularly the *psychologic problems* posed by *potentially fatal disease*. The physician who refuses to acquaint his patient with the diagnosis of a hematologic malignant disease may be protecting himself, rather than his patient, from emotional trauma. Only rarely are the circumstances such that informing the patient is best avoided. A patient who must undergo the type of therapy employed realizes that the disease is serious. Unless he has meaningful knowledge of his disease, a distressing syndrome may develop; he knows that he is seriously ill, but realizes that his family (and physician) do not want to talk about it. As a result, he is denied the emotional support of full and frank discussion and is isolated from meaningful communication with his

loved ones at a time when he most needs it. *However, the discouraging information should be presented as kindly as possible, even though it should be truthful.* Certain studies<sup>177</sup> suggest that teenagers and even younger children are relieved by acknowledgement of the diagnosis and discussion of its implications. The responsibility imposed upon the physician in informing the patient is considerable, but must be accepted. In most instances the hematologist is a consultant who has had no previous acquaintance with the patient or his family. Consequently, he is faced with communicating information to a person with whom he is, at best, minimally acquainted.

One must determine, by subtle clues, when the patient wants to talk about his disease. Often the patient will take the initiative by asking the physician if a diagnosis has been made. If the physician answers in the affirmative, but then counters by inquiring what the patient thinks his disease is, it is surprising how frequently the patient will reply that he thinks he has leukemia. Thus, the barrier is broken.

Leukemia carries a greater reputation as a "dreaded disease" than do other forms of hematologic malignant disease, probably because of the prognosis associated in the minds of laymen with the acute leukemias. If the diagnosis is leukemia, it is wise to determine what the patient knows about leukemia. If the diagnosis is anything other than AML, the physician then can explain that there are various types of leukemia, that prognosis differs with each, and that the patient does not have one of the worse varieties. Conversely, if enlarged lymph nodes are due to HD or other lymphomas, it should be explained that the patient does not have leukemia, but rather has a tumor of lymph nodes. The patient often wants to know if his disease is a cancer. Most patients and many physicians equate cancer with carcinoma and it is wise to explain that while some consider the lymphomas to be a form of malignancy, their behavior and their therapy are quite different from those of other forms of cancer. These types of interchanges provide the background against which one

can go on to discuss the many problems posed by the disease and its management.

Whether there is a reasonable chance of cure should be discussed realistically. That there may be a slight chance of cure should never be completely ruled out in discussion with the patient; there is no malignant neoplastic disease, no matter how extensive, in which apparent recovery has not been reported although recovery may be an extremely remote possibility. Thus, with stage I, II, or even III HD, cure following extensive therapy should be presented as probable, but by no means certain; while, with AML, cure can be presented as quite unlikely, but not impossible. Such a discussion is by no means in contradiction with the physician's therapeutic approach (page 1703) and allows the patient to retain hope.

It is unwise to tell the patient anything concerning average survival rates for his disease. Such averages are valuable to the physician in comparing different modes of therapy, but are derived from data that have such a wide or skewed distribution that they have little meaning with reference to the individual patient. It is not dishonest to mention one's long survivors with the disease in question, as long as one is careful to emphasize that similar survival is a possibility and not an expectation. If patients maintain some hope, their outlook usually is more optimistic and their life becomes more rewarding than when they are led to regard their diagnosis as a death sentence with finite limits and no hope.

The physician must be prepared not only to discuss all aspects of the patient's disease, but must stimulate the patient to hold free and frank discussions with him. It is helpful for the physician to encourage the patient repeatedly to ask questions no matter how trivial the questions seem to be. Most commonly, the patient is concerned about what changes in his usual life are necessary. Since there is no good evidence that diet, drink, or physical and other accustomed activities modify the course of any of the diseases under consideration, the best advice to give the patient is: "Lead as normal a life as possible and do what you feel like doing." This

requires repetition to be believed. It is also advisable to warn the patient that relatives, friends, and neighbors are likely to give well-meant, but often faulty, advice. In general it is best to limit the number of friends and associates who are informed about the diagnosis. Although there are patients who transfer transiently or permanently to the "cancer quacks," the frequency of such transfer is markedly reduced if free and frank discussion with the patient and his close relatives is practiced.

With regard to therapy, it is important to explain what the goals are—curative or palliative. Similarly, patients should be warned concerning the side effects of the drugs used and the complications of their disease, without being led to expect that any or all of these will occur. If patients feel free to call their physician when they are concerned about a problem, proper care is facilitated. If the concern is minor, reassurance relieves the patient and conserves the physician's time. If the problem is of importance (ie, development of fever in CLL), prompt diagnosis is facilitated and the well-being of the patient and the effectiveness of the physician are enhanced.

It is of value to discuss research concerning the cause and treatment of the disease with the patient. Hope for a cure of the incurable is an important ingredient and such hope can be justified by on-going research. If the patient can be assured, truthfully, that his physician will be aware of any new developments relating to his disease, some degree of comfort is provided and the exaggerated reports of new forms of therapy that reach the news media are more easily explained.

The death of a child is particularly traumatic psychologically<sup>24</sup> and much care and counseling must be provided the patient and the parents of children with leukemia and other tumors.<sup>54</sup>

In summary, the physician must deal with the patient and the patient's family individually rather than as another "case." If he cannot cure, he must at least bring all his skills to bear to provide comfort.

Prolonging misery as compared to prolonging enjoyable life is a subject that is often

discussed by thoughtful physicians, but is rarely considered in print in a realistic fashion. The physician often realizes that, in all of the diseases under discussion, a stage is reached ultimately when there is little chance of helping the patient to achieve any degree of comfortable life. Yet, by means of various drugs and complicated life support systems he knows that he can probably sustain a semblance of life for days, weeks, or even months. There are physicians who believe that life must be sustained for as long as possible under any circumstance. Others, such as ourselves, think that the long-continued absence of some degree of comfort and ordinary enjoyment of life ceases to meet the definition of life. Positive acts to terminate life in such circumstances constitute euthanasia, but failure to act with therapeutic vigor is a different matter. With respect to the failure to react vigorously to an apparently hopeless situation, the accord, often unspoken, reached between physician, patient, and patient's family often is remarkable. However, the physician bears the responsibility for reaching a decision as to whether or not to use every possible means to maintain the patient's life for a few more days and cannot put the burden of this decision solely on the family. When a firm decision to resist further therapy has been reached by a patient, the widely variable psychologic reactions of members of the medical staff may interfere with their ability to serve the best interests of the patient.<sup>155</sup> And lastly, the dignity of the patient must be respected and the trauma to his loved ones caused by the sight of a body entrapped by tubes and other disagreeable equipment must be avoided unless something really worthwhile is to be gained by use of such devices.

### Agents Useful in the Treatment of Hematologic Neoplasms<sup>122,123,153,197</sup>

**HISTORY.** Unlike most forms of carcinoma, *surgical excision* has never played a prominent role in the therapy of the leukemias, lymphomas, or myelomas. As discussed in Chapters

50 and 51, surgical removal of local foci of HD or NHL has resulted in occasional apparent cures, but extensive dissection and removal of diseased tissue has not proved to be of benefit. Early enthusiasm (1900-1910) for cure of HD by radiotherapy<sup>93</sup> was followed by a half century of pessimism; radiotherapy came to be considered a useful palliative procedure, but in no sense a curative measure. With technical improvements, radiotherapy has been reaffirmed as potentially curative (see Chapter 50). Similarly, when the first complete remissions were induced with antifolic acid chemotherapy in AL in 1947-1948, the hope that these antimetabolites were curative was quickly negated by rapid relapse of disease. Modes of therapy developed subsequently, such as use of asparaginase, have been associated with similar phases of initial enthusiasm followed by discouragement. Ultimately, however, apparent consistent cure of a tumor by chemotherapy was demonstrated by the use of toxic doses of methotrexate in choriocarcinoma. That this tumor, which contains non-host tissue (male contribution to gene constitution), was curable by chemotherapy did much to establish the validity of nonsyngeneic animal tumor transplant systems as models for nonsyngeneic tumors. At the same time, the fact that most human tumors arise strictly from the host raised doubt concerning the validity of transplanted, nonsyngeneic tumors in animals as models for the evaluation of chemotherapeutic agents.<sup>163</sup> However, the cure of syngeneic human tumors by chemotherapy appears to have been accomplished in a significant number of patients with Burkitt's lymphoma (Chapter 51) and perhaps in those with HD (Chapter 50).

### Classes of Antitumor Agents

The physical agents that are of general usefulness as antitumor agents are x- and gamma-irradiation, but beta- and electron-beam irradiation also are of potential benefit. These and the chemotherapeutic agents that are of proven value in the treatment of the hematologic malignant diseases are listed in Table 55-1.

**Table 55-1. Agents Useful in Therapy of Hematologic Malignant Diseases**

Agent	Non-Cell-Cycle Dependent	Cell-Cycle* Dependent
Irradiation		
X-rays	✓	
γ-rays	✓	
Alkylating agents		
Nitrogen mustard	✓	
Chlorambucil	✓	
Cyclophosphamide	✓	
Busulfan	✓	
Melphalan	✓	
Antimetabolites		
Methotrexate		✓
6-Mercaptopurine		✓
Cytosine arabinoside		✓
Hydroxyurea		✓
Stathmokinetic agents		
Vinblastine		✓
Vincristine		✓
Antibiotics		
Daunomycin		✓
Bleomycin		✓
Adrenal glucocorticosteroids	✓	
Miscellaneous agents		
L-Asparaginase	✓	
Procarbazine	✓	
Nitrosoureas		✓?
Dibromomantrol	✓?	

\*The drugs listed in this column are more effective in a particular stage of the cell cycle than in another. Nevertheless, the action of all agents may be cycle dependent to some degree.

The effectiveness of physical and chemotherapeutic agents in killing tumor cells depends, at least in part, on what stage in the generative cycle of cell growth most of the tumor cells are to be found. As discussed in Chapter 2, the generative (G) cycle can be divided into G<sub>0</sub>, G<sub>1</sub>, S, G<sub>2</sub>, and M phases. Cells in G<sub>1</sub> will progress within a finite time into DNA synthesis (S), then to G<sub>2</sub>, and then divide by mitosis (M). Thus, cells in G<sub>1</sub>, S, G<sub>2</sub>, and M constitute the proliferative compartment. Cells in G<sub>0</sub> are nonproliferating, but can be stimulated to enter G<sub>1</sub> by appropriate, but poorly defined, stimuli. A classification of chemotherapeutic agents as "cycle dependent," or not, is useful in applying cell kinetic considerations in the design of therapeutic regimens (page 1709).

Agents that are "cycle dependent" are those that do not kill cells in  $G_1$  or  $G_0$ , but selectively kill cells in S or M. Noncycle-dependent agents are those that kill in  $G_1$  or  $G_0$  (interphase death) as well as in S,  $G_2$ , or M. This distinction, however, is not sharp. Thus, some noncycle-dependent drugs have a greater effect on cells in cycle than on those in interphase (cyclophosphamide<sup>176</sup>), whereas others may be less effective on cells in S and  $G_2$  than in other phases of the cycle (HN2<sup>9</sup>).

The diseases in which each of the various drugs has been shown to be beneficial are summarized in Table 55-2. Detailed recommendations regarding the use of these agents

will be found in Chapters 47 to 53. It is evident that no single agent is of benefit in all diseases and that no disease is modified beneficially by all available agents (Table 55-2).

The *ideal drug* is one that kills tumor cells, but has no effect on normal cells. While no such agent has been found, significantly greater killing of tumor as compared to normal cells is exhibited by four agents: steroids, asparaginase, vincristine, and bleomycin. Other agents may have a slightly greater effect upon tumor cells than upon normal tissue, but their tumor-killing effects are more dependent upon differences in the ki-

Table 55-2. Effectiveness of Agents in Various Hematologic Neoplasms

Agent	Hodgkin's Disease	Lymphosarcoma	Reticulos Cell Sarcoma	Chronic Myelocytic Leukemia	Chronic Lymphocytic Leukemia	Acute Myeloblastic Leukemia	Induce Remission	Acute Lymphoblastic Leukemia Maintain Remission	Multiple Myeloma
Irradiation	+	+	+	+	+	(+)	-	(+)	+
Alkylating agents									
Nitrogen mustard	+	+	+	+	+	-	-	?	-
Chlorambucil	+	+	+	+	+	-	-	?	±
Cyclophosphamide	+	+	+	+	+	-	-	?	+
Busulfan	?	?	?	+	+	-	-	?	?
Melphalan	±	±	±	+	+	-	-	?	+
Antimetabolites									
Methotrexate	+	+	±	±	±	±	+	+	?
6 Mercaptopurine	?	±	+	+	?	+	+	+	?
Cytosine arabinoside	?	?	?	?	?	+	+	?	?
Hydroxyurea	+	+	?	+	?	?	?	?	-
Stathmokinetics									
Vinblastine	+	+	+	?	?	±	±	?	?
Vincristine	+	+	+	?	?	±	+	-	?
Antibiotics									
Daunomycin	+	?	?	?	?	+	+	?	?
Bleomycin	+	+	+	-	-	-	-	?	?
Adrenal glucocorticosteroids	+	+	±	-	+	-	+	-	±
Miscellaneous									
L-Asparaginase	?	?	?	?	?	±	+	-	?
Procarbazine	+	+	+	?	?	?	?	?	±
BCNU	+	+	+	?	?	?	-	?	?
Dibromomantol	?	?	?	+	?	?	?	?	?

Symbols used for effect on disease are: +, induces general improvement, (+) useful for selected complications only ± some evidence for favorable effect, but of limited value -, no favorable effect, ?, inadequate clinical trials

netics of recovery of tumor and of normal cell populations than on their less damaging effect on normal cells (see below).

### Normal and Tumor Cell Kinetics as Related to Antitumor Therapy

At one time it was assumed that tumors grow more rapidly than do comparable normal tissues. As regards neoplastic disease of hematopoietic tissue, this hypothesis has been

largely disproved; eg, in the acute leukemias or in CML, the generation time for the average potentially dividing cell tends to be longer than normal<sup>167,98,112,123</sup> (also see Chapter 46). Consequently, even if normal hematopoietic cells are killed to the same extent as tumor cells, recovery of normal cells will be faster (Fig. 55-1). This idealized concept of tumor cell kill is based on the extensive studies of investigators such as Skipper and colleagues in murine systems.<sup>160,162,163</sup>

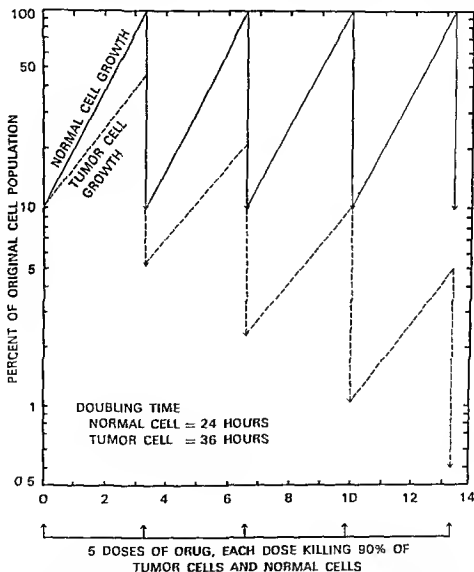


Fig 55-1. Differential effect of a noncycle-active drug on normal hematopoiesis and on tumor cells based on differences in growth rate of the two systems. Tumor growth rate is slower than that of normal hematopoietic tissue so that while each dose of a drug (or irradiation) will destroy an equal proportion of the total cell mass, tumor size is progressively reduced (From Boggs and Chervenick,<sup>20</sup> courtesy of the authors and Grune & Stratton)

A fixed percentage of a sensitive cell population is killed by each dose of a drug or physical agent.<sup>78</sup> With noncycle-dependent agents the percentage of the population killed increases as the dose of the agent is increased so that exponential killing is observed. The classic model for this type of cell kill is irradiation (Fig. 55-2). With cycle-dependent drugs, only that segment of the cell population that is in the sensitive phase of the cycle is affected. Thus, when drugs such as methotrexate or cytosine arabinoside are given to patients in whom a limited proportion of the population of tumor cells is in cycle, a plateau of killing is reached (Fig. 55-3). Current data on tumors in man, such as ALL,<sup>112,129</sup> suggest that most of the tumor cell population is in  $G_0$  or in a prolonged  $G_1$ . Thus, most of the population is not affected by a single

dose of a cycle-dependent agent; repeated therapy is required. As a portion of the compartment is killed with each dose, it is assumed that recruitment into cycle is induced in the residual population, making more cells available for the action of the drug (Fig. 55-4). The use of specific cycle active drugs at a time when cells are wholly or partially synchronized in the sensitive phase of the cycle may lead to distinct improvement in the therapeutic efficiency of such drugs.<sup>101</sup>

From such kinetic considerations the concept of "total cell kill" as a therapeutic approach has arisen; this concept is similar to that of the surgical approach of total excision of tumor tissue. In murine transplanted tumor systems, in which an accurate measure of the total number of tumor cells in the host can be obtained, killing curves resulting in

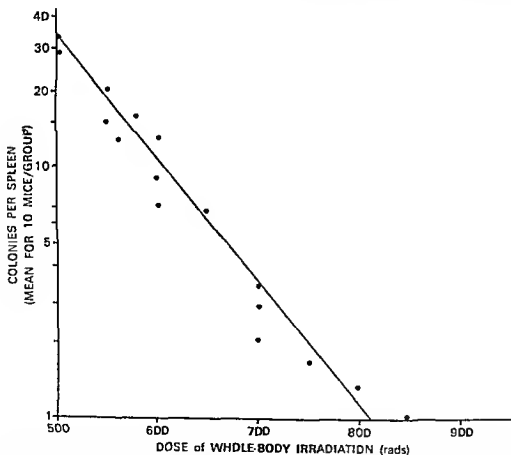


Fig 55-2. Effect of increasing doses of a noncycle-active agent (whole-body irradiation) on the number of endogenous murine hematopoietic spleen colonies. Each colony represents the survival of a pluripotential hematopoietic stem cell. The killing of such cells is an exponential function of dose. (Courtesy of SS Boggs.)

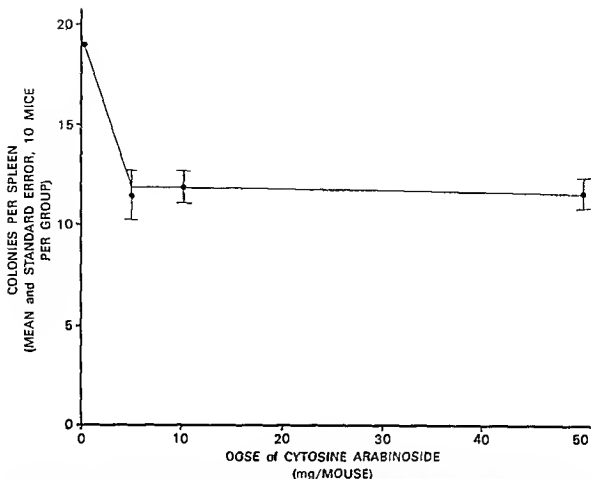


Fig 55-3. Effect of increasing doses of a cycle-active agent (cytosine arabinoside) on transplanted spleen colony-forming cells. Each colony represents the survival of a pluripotential hematopoietic stem cell. As the dose is increased, a plateau of killing results, indicating that most of the cell population is in a long  $G_1$  or in  $G_0$ . (Courtesy of SS Bogggs)

total cell kill have been published.<sup>163</sup> In human tumors the total number of tumor cells at the start of therapy cannot be measured and, while calculations have been made, particularly in ALL,<sup>84</sup> the number of assumptions involved in such calculations is so large that their accuracy is doubtful. However, accomplishment of total cell kill in human tumors in certain circumstances is suggested by indirect evidence. For example, in HD, the chance of recurrence in an irradiated area is a negative exponential function of the irradiation dose delivered to that area (see Chapter 50). The probability of total cell kill in an exponential killing system can approach, but never reach, 100%.

Ideally, one would like to test the patient's

tumor cells against a variety of agents in an *in vitro* system, thereby predicting the most effective agent for each patient.<sup>40</sup> However, this desirable goal has yet to be attained.

#### Interaction of Various Therapeutic Agents

Cross resistance and possible antagonism between agents are important concepts to consider in choosing the best form of therapy for each patient.

Cross resistance has been demonstrated clearly for a number of agents (Table 55-3)<sup>164</sup> and certain generalizations seem justified. The patient who is not or is no longer responsive to one alkylating agent is unlikely



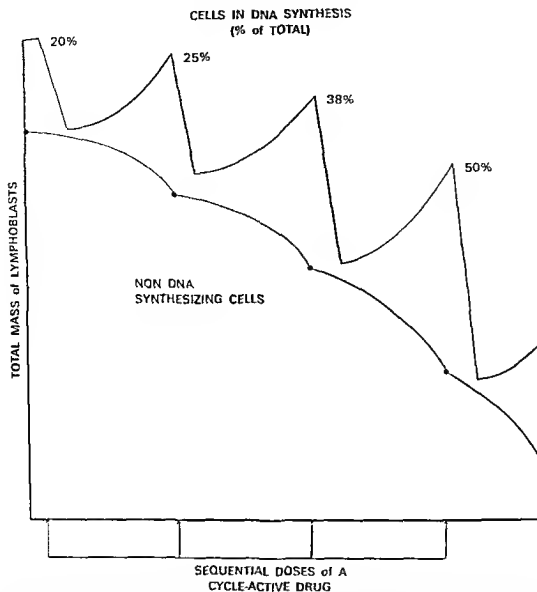


Fig 55-4 Effect of repeated doses of a cycle-dependent agent on killing of lymphoblasts in leukemia and recruitment into cycle from  $G_0$ . With each dose the compartment is reduced by the number of cells that were in the sensitive phase of the cycle and in response to compartment depletion, a greater proportion comes into cycle after each dose. This somewhat idealized figure is derived from studies such as those of Lampkin et al.<sup>112</sup>

to be responsive to another alkylating agent. Similarly, resistance to one of a class of closely related agents would imply resistance to another in that specific group; it seems likely that resistance to 6-MP implies resistance to similar purine analogs and that resistance to methotrexate implies resistance to all other compounds that act as inhibitors of folic acid. Conversely, there is no evidence

for significant antagonism within a class of agents. For example, in ALL there is abundant evidence that initial response to one agent, such as methotrexate, does not reduce the likelihood of subsequent response to another agent such as 6-MP (Chapter 47).

Neither synergism nor antagonism has been shown between drugs in any tumor system in man, but the absence of such evi-

**Table 55-3. Cross Resistance of Chemotherapeutic Agents**

<i>Agent Useful in Treatment of Hematologic Neoplasm</i>	<i>If Resistance to Agent Develops, Resistance to the Following Agent is Expected*</i>
Adrenal glucocorticosteroids	None
Nitrogen mustard	Cyclophosphamide, chlorambucil
Cyclophosphamide	Nitrogen mustard, busulfan
BCNU	Cyclophosphamide
Methotrexate	Thioguanine
6-Mercaptopurine	Thioguanine, azaguanine
Vincristine	Vinblastine
Asparaginase	None
Procarbazine	Unknown
Hydroxyurea	Unknown

\*This list is limited to instances of proven cross resistance. The absence of an agent from the list does not mean that cross resistance does not occur. Adapted from Skipper et al.<sup>144</sup>

dence does not deny this occurrence, since direct measurement of the number of cells killed has not been possible. Indirect evidence, such as rate of remission in acute leukemia, is the only available criterion. Examination of the results of clinical trials of drugs used in combination for inducing remission in ALL, as compared to predicted responses if the effects of the drugs were simply additive in their action, fails to suggest either synergism or antagonism in the aggregate (Table 55-4). One might suggest that synergism is present between daunomycin and prednisone and that there is antagonism between 6-mercaptopurine and methotrexate. However, favorable or unfavorable modification of toxicity is as reasonable an explanation for the differences in the observed effects as is synergism or antagonism. Furthermore, the identity of the average figures of predicted and observed values for all combina-

**Table 55-4. Synergism or Antagonism between Antitumor Agents in Inducing Remission in Acute Lymphoblastic Leukemia**

<i>Average Response to Single Agents*</i>		<i>Predicted Additive Response to Combination†</i>	<i>Observed Response*</i>
Prednisone	69%	91%	89%
Vincristine	72%		
Prednisone	69%	88%	91%
6-Mercaptopurine	60%		
Prednisone	69%	79%	81%
Cyclophosphamide	31%		
Prednisone	69%	79%	92%
Daunomycin	32%		
6-Mercaptopurine	60%	70%	58%
Methotrexate	25%		
Prednisone	69%	97%	88-94%
Vincristine	72%		
6-Mercaptopurine	60%	84%	84%
Methotrexate	25%		
Total Average			

\*Data from Table 47-6, complete plus partial remission

†Calculated with the assumption that each drug acts in an additive fashion and affects an independent proportion of the population, the probability of not responding to one drug was multiplied by the probability of not responding to another. For example, the probability of not responding to prednisone is 0.31, for vincristine, 0.28, and the probability of responding to neither is 0.09.

tions examined suggests that drugs used in combination act in a simple additive fashion (Table 55-4).

### Hematopoietic Toxicity of Antitumor Agents

There are significant differences in the toxic manifestations of all of the agents used in the treatment of hematologic malignancies. The toxic effects of each agent, whether dose related or idiosyncratic, will be discussed subsequently. However, it is the dose-related effects of most agents upon the normal hematopoietic system which constitute the most common toxic manifestations that require interruption or modification of therapy. Other toxic effects may be just as common but not as easily detected, eg, reversible aspermia or oligospermia has been noted following chemotherapy of AL<sup>155a</sup> and lymphoma<sup>159a</sup> and after irradiation in HD.<sup>166a</sup> This is true of all agents except for prednisone and bleomycin, which appear to be free from hematopoietic toxicity, and vincristine and asparaginase, which have no such toxicity in most patients but induce severe hematopoietic depression in a few.

No significant direct effect is observed on mature cells of the blood or upon the post-mitotic compartments of the marrow, with the possible exception of such subtle effects as reduced phagocytic killing by neutrophils exposed to x-irradiation.<sup>157</sup> It is the proliferating and potentially proliferating marrow precursors of neutrophils, platelets, and erythrocytes, as well as those of the immune system (lymphocytes), that are affected. Consequently, knowledge of normal cell kinetics, as outlined in earlier chapters, is important in predicting and understanding hematopoietic toxicity, particularly in its recognition by the examination of changes in blood cells.

From studies in the mouse,<sup>19,20</sup> it is assumed that the pluripotential hematopoietic stem cell compartment, which gives rise to the myeloid elements, is normally a cell compartment primarily composed of noncycling cells. Consequently, cycle-dependent drugs have less effect on this compartment than do

noncycle-dependent drugs. There is evidence in the mouse to suggest that busulfan damages the pluripotential stem cell compartment to a greater degree than it does the more mature compartments.<sup>19</sup>

Thus, certain predictions can be made concerning hematopoietic toxicity, as reflected in blood values for single (Fig. 55-5) or repeated (Fig. 55-6) doses of chemotherapeutic agents.

A single dose of an agent that destroys all hematopoietic cell production, ie, a potentially lethal dose of whole-body irradiation or cyclophosphamide, spares only mature and post-mitotic maturing cells of the blood and marrow. Thus, following administration of such an agent, blood values would reflect storage pools of cells and cell life span only. In the case of platelets, for which little or no storage reservoir exists (Chapter 9), the number of these cells in the blood would decline steadily and the cells would disappear in approximately 10 days—the limit of platelet life span (Fig. 55-5). Neutrophils would be maintained in normal concentration until the post-mitotic storage and maturation pool of the marrow is exhausted (approximately 10 days) (Chapter 6); then their concentration would fall precipitously owing to their short intravascular sojourn (Fig. 55-5). In contrast, the long life span of erythrocytes allows near normal values to be maintained beyond the time when severe neutropenia and thrombocytopenia have developed. In treating a patient, the onset of cytopenia can be expected to be earlier than depicted in Figure 55-5 since the patient's disease may have modified the system so that the neutrophil storage pool is reduced while treatment and infection may have induced inflammation, resulting in increased cell utilization.

In contrast to a drug that destroys recognized mitotic compartments as well as noncycling stem cell compartments (Fig. 55-5), a single dose of a cycle-active agent may destroy all recognized mitotic compartments, but the noncycling stem cell compartment is spared. In such instance, the marrow can regenerate and begin to replace mature cells before complete pancytopenia has been induced.

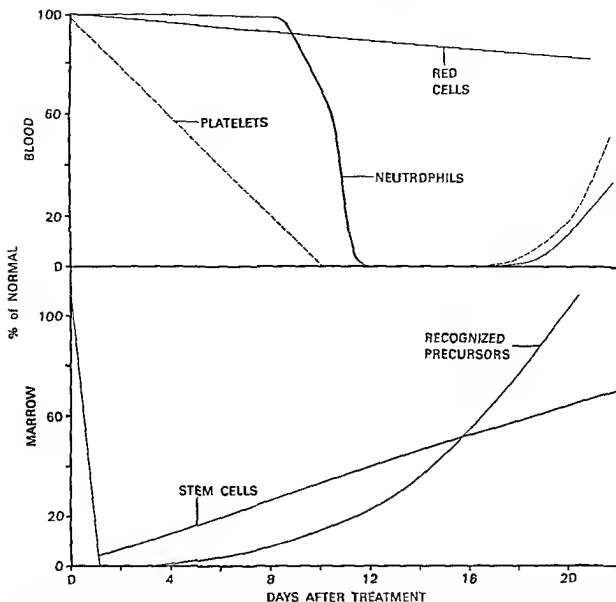


Fig 55-5. Effects of a potentially fatal dose of a drug on blood cell level and marrow production. With a potentially lethal dose, recovery of the stem cell and of the mitotic compartments is delayed so long that severe pancytopenia occurs. Rate of loss of mature and maturing post-mitotic cells is based on normal cell kinetics as reviewed in Chapters 5, 6, and 9 (Schematic)

However, chemotherapeutic drugs are not ordinarily administered in single doses. The effect on neutrophils, platelets, erythrocytes, and stem cells of regularly repeated doses is much more complex than that of single doses. The action of repeated doses of a DNA-inhibiting cycle-active agent upon the stem cell system, the marrow mitotic pool, the marrow storage pool, and the blood neutrophil compartment is shown in Figure 55-6.

The postulated dose of drug is the amount that is sufficient to destroy any cells that are in DNA synthesis. It is assumed that DNA synthesis accounts for one half the cycle.

With the first dose, the stem cell compartment is reduced only very slightly, since only a small proportion is in DNA synthesis. The marrow mitotic pool is reduced by half, since half of it is in DNA synthesis. Neither the marrow storage pool nor the blood is imme-

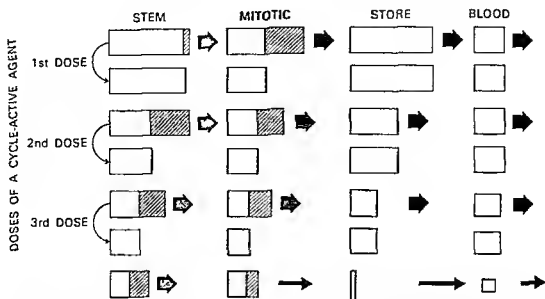


Fig 55-6 Effects of repeated doses of a cycle-active agent on neutrophils in the blood and in the marrow storage compartment and upon stem cell and recognized mitotic compartments. See text for further description. (From Boggs and Chervenick<sup>20</sup> courtesy of the authors and Grune & Stratton.)

diately affected. By the time the second dose is administered, the stem cell pool has hypertrophied slightly in response to the damage in the more mature compartments; half of it is now in DNA synthesis, and with the second dose half of it is destroyed. Again the marrow mitotic pool is reduced by half, and, since it had not fully regenerated, it is smaller than after the first dose. The marrow storage pool again is not affected by the second dose, but is smaller than normal because of reduced feed-in from the marrow mitotic pool and continued output to the blood. Since significant storage remains, the blood has not been affected. By the time the third dose is given, some regeneration has occurred in the stem cell compartment, but the stem cell pool has not returned to normal and half is still in DNA synthesis. Therefore, it is again reduced by half and the same is true for the marrow mitotic pool. Again, because of continued normal feed-in to the blood and subnormal feed-in from the marrow mitotic pool, the marrow storage pool is further reduced and is now rather small. However, it is still maintaining the blood pool at a near-normal size. By the time the next dose is due,

disaster has struck. There is severe neutropenia because the storage pool has become completely exhausted and feed-in into the blood has been markedly reduced.

It seems reasonable to assume that delayed and somewhat unpredictable hematopoietic toxicity associated with drugs such as BCNU (page 1730) reflects a selective damaging effect of the drugs on resting hematopoietic stem cells.

It must be emphasized that changes in numbers of blood cells lag significantly behind changes in the precursor compartments that are the sites of action of antitumor agents. Thus, while we use the blood cell level as a guide to hematopoietic toxicity, we must recognize that toxicity is already relatively severe when changes in the numbers of blood cells are evident.

In addition to quantitative changes in blood cells, qualitative morphologic changes frequently are produced. Megaloblastosis is commonly observed; drugs that selectively affect DNA synthesis, such as methotrexate, 6-MP, and cytosine arabinoside, frequently are associated with the appearance of megaloblasts. The general mechanism is presumably

the same as that resulting in megaloblastosis in  $B_{12}$  and folate deficiency (Chapter 14); nuclear (DNA) synthesis is retarded in growing cells, while synthesis of cytoplasmic constituents is affected to a lesser degree or not at all.

As noted in Chapter 54, therapy may cause suppression of immune function. When intensive therapy of ALL was stopped, repopulation of lymphoid tissues was found to occur more rapidly in younger than in older children.<sup>25a</sup>

## Specific Antitumor Agents

The following discussion of the dosage, route of administration, mechanism of action and toxicity of agents useful in treating hematologic neoplasms is necessarily brief. More detailed discussions will be found in published reviews.<sup>29,34,122,123,153,197</sup>

### Radiotherapy<sup>96</sup>

In 1902, shortly following the discovery of x rays, Pusey<sup>148</sup> described the usefulness of these rays in treating patients with HD and those with other lymphomas. The value of x rays and the closely related gamma and beta rays emitted by various radioisotopes has been amply confirmed.

The dose of radiation delivered to a tumor or to surrounding normal tissue is best measured in rads rather than in roentgens. "Rad" refers to the actual absorbed dose in tissue, while "roentgen" is a measure of ionization in air. The effective dose of x rays not only depends upon the number of rads delivered to tissue, but also upon the rate of delivery. The rate of delivery and the penetrance of the x rays are functions of the energy (voltage) of x-ray machines and the type of filtration to which the generated beam is subjected. Modern radiotherapy machines operate in megavoltage ranges and have almost entirely supplanted kilovoltage equipment for most purposes in most treatment centers. Van de Graeff electrostatic generators of x rays have a peak energy of approximately 2 million electron volts (MeV); radioactive

cobalt teletherapy units emit gamma rays with energy equivalent to 3 MeV, and linear electron accelerators and betatrons, machines capable of generating either gamma or x rays, provide x-ray beams with as high as 8 and 40 MeV, respectively.

Selection of the desired total tumor dose and of its delivery time is empiric and, as was discussed in Chapter 50, depends in large part upon whether the therapeutic intention is curative or palliative. Thus, 4000 rads delivered within four to six weeks may be desirable in radical radiotherapy of HD (Chapter 50), 2400 rads is a desirable total dose to the craniospinal axis as prophylaxis against development of CNS leukemia (Chapter 54), while doses of only 400 to 600 rads to the spleen of patients with CML may produce the desired effect (Chapter 48). Optimal dose schedule with respect to dose fractionation has not been clarified for any human tumor.<sup>53</sup>

X rays or gamma rays generated by megavoltage equipment are highly penetrant and low-energy beams generated by such equipment can be largely eliminated by placing filters of copper or aluminum in their paths. Thus, deep-seated tumors can be treated with high doses without inducing intolerable damage to overlying tissues such as the skin; such damage was the primary dose-limiting factor with kilovoltage equipment.

Shielding of normal tissue, treatment of large fields to avoid the problem of calculating doses delivered to areas overlapped by small fields, and a variety of important technical aspects of radiotherapy are crucial considerations in the proper use of radiotherapy.<sup>96</sup>

Radioactive isotopes may be used as external sources of irradiation in machines in which cobalt ( $^{60}\text{Co}$ ) or cesium ( $^{137}\text{Cs}$ ) constitute the energy source. They may be injected into the patient as localized implants of radium or may be given systemically with the knowledge that the isotope tends to localize in certain areas. Thus, radioactive sodium phosphate ( $^{32}\text{P}$ ) localizes in bone marrow, colloidal radioactive gold in the reticulo-endothelial system, and radioactive strontium in bone.

The gamma rays emitted by isotopes such as  $^{32}\text{P}$  have limited penetrance and their beta rays even less. Thus, irradiation is confined to a very limited area surrounding the site of lodgement of the administered isotope. In therapy with  $^{32}\text{P}$ , most of the isotope is incorporated into dividing hematopoietic cells and production in this compartment is reduced with minimal radiation damage occurring in other tissues. Although this isotope has found some use in treating CLL and CML (Chapters 48 and 49) its most common use has been in the treatment of polycythemia vera (Chapter 30).

Other low-energy rays include *electron beams*, whose low penetrance makes them useful in treating skin diseases such as mycosis fungoides (Chapter 51).

### *Effect of Ionizing Radiation on Cells*<sup>7,24,25,53,96,115</sup>

Ionizations resulting from the passage of x or gamma rays, or charged particles, through or into cells may lead to disruption of biologically important macromolecules either directly or by production of free radicals. These free radicals are exceedingly unstable molecules with unpaired electrons and, thus, can combine with and damage or inhibit normal cellular constituents.<sup>69</sup> The events that follow depend on the total number of ionizations (dose), rate of ionization (dose rate), type of cell, phase of cell cycle, and ability of the cell to repair or replace damaged molecules. The unrepaired damage may result in instant death of the cell during irradiation (about 100,000 rads) together with gross alterations of the chemical machinery of the cell with coagulation of proteins and depolymerization of DNA. With much smaller doses (100 to 1000 rads), death is delayed. Delayed death may occur in interphase before and independent of DNA synthesis or mitosis, during DNA synthesis or mitosis, or after one or more mitoses have occurred. Permanent inhibition or severe retardation of mitosis, with the cell surviving but productively dead, can occur. The likelihood of cell death may be related to the stage of the generative

cycle (Chapter 2), in which the cell is found at the time of irradiation exposure. Tumor cells irradiated *in vitro*<sup>52</sup> or pluripotent, hematopoietic murine stem cells irradiated *in vivo*<sup>23</sup> are more affected by irradiation while in  $\text{G}_1$  or  $\text{G}_0$  than during DNA synthesis.

*Radiation protection*<sup>96</sup> may be achieved by physical shielding or by the use of an agent that either absorbs some of the energy of ionization, reacts with free radicals, or alters the biologic target so that it is less readily damaged. Among the most effective of such agents are the sulfhydryl-containing aminoethiols that are strong reducing agents at cellular temperature and pH. Reduced  $\text{O}_2$  will also protect cells. All these agents have the effect of reducing the radiation dose; they must, of course, be present at the time of exposure.

Certain treatments given to exposed mammals after irradiation can protect the animals from death by accelerating the replacement of damaged cells in critical tissues without changing the initial damage produced by x rays. For example, enhanced survival of mice receiving whole-body irradiation can be achieved by injecting a variety of substances, such as endotoxin, before irradiation or by bleeding the animals before or immediately after irradiation.<sup>19</sup> In these circumstances the cells are not protected individually from irradiation, but, rather, stem cell compartments are either expanded in size or stimulated to divide more rapidly. As a consequence, post-irradiation recovery of hematopoiesis is accelerated and death due to neutropenia or thrombocytopenia is avoided.

All cells are subject to damage by irradiation, but their sensitivity varies from organ to organ and from species to species. Single exposure of animals to whole-body irradiation (WBI) results in death at certain predictable doses. For example, mice given 5000 or more rads of WBI will die within a few hours of apparent x-ray damage to the brain cells. Given 1200 rads, they will die in three to six days from apparent x-ray damage to the gut and, given 900 rads, they will live for eight to fifteen days and die of infection or bleeding due to pancytopenia secondary to hemato-

poietic damage (page 1714). The dose of WBI resulting in hematopoietic death in man is probably 300 to 500 rads. The "gut death" and "cerebral death" doses of WBI in man have not been approximated with any accuracy. As an example of extreme species variability, one may cite the fact that the shark's brain is not damaged significantly until doses in excess of 30,000 rads are given.<sup>144</sup>

Insofar as has been determined with clarity, all effects of irradiation are the direct result of damage to the cells exposed to irradiation. *Abscopal effects*, effects of irradiation on organs that are not irradiated but are injured as the result of irradiation of a distant organ, have often been postulated, but have never been proved. Most studies dealing with possible abscopal effects consisted of investigation of effects on the hematopoietic system, such as general bone marrow depression following repeated irradiation to the spleen.<sup>21</sup> Since it is now known that the circulating and migratory pathways of hematopoietic stem cells include the spleen (Chapter 2), the results of such studies may be explained by irradiation of stem cells rather than being due to true abscopal effects.

### Toxic Effects of Radiotherapy

The toxic effects of radiotherapy can be divided into complications occurring during therapy, those occurring within a few months of completing therapy, and remote complications. The remote complications generally are limited to a higher incidence of AML and of certain other forms of malignancy in irradiated patients than in those not irradiated, and were discussed in Chapter 46.

The *hematopoietic system* (page 1714), the gastrointestinal tract, and the skin are most often affected during radiotherapy; the toxic effects in these tissues are usually reversible when therapy is stopped.

The epithelium of the entire *gastrointestinal tract* is a rapidly renewed cellular system and is quite sensitive to irradiation.<sup>96</sup> Thus, depending upon the area irradiated, symptoms and signs of gastrointestinal damage are common. The throat and oral cavity may

become inflamed and even ulcerated, producing pain and dysphagia. Esophagitis may occur. Irradiation of the stomach regularly induces anorexia and nausea, and vomiting often occurs. Irradiation of the small and large intestine may result in malabsorption and diarrhea and, in extreme cases, bloody diarrhea may occur, a serious and sometimes fatal complication. Residual evidence of damage to the gut following recovery from acute radiation toxicity is unusual.

The *skin* becomes erythematous and tender. Before the introduction of megavoltage equipment, severe inflammation of the skin progressing to desquamation and ulceration was the primary factor limiting the dose. Even with megavoltage rays, some erythema is common, as is permanent loss of body hair and hyperpigmentation of skin in irradiated areas. Telangiectatic lesions may be produced. Subcutaneous fat necrosis, followed by subcutaneous fibrosis, is rare after a single course of radiation therapy, but occurs in areas that have been treated on more than one occasion.<sup>96</sup>

More serious effects of irradiation that may not become apparent until a course of irradiation has been completed most often are due to damage to lung, pericardium, kidney, and liver.

**RADIATION PNEUMONITIS AND FIBROSIS OF LUNG.**<sup>96</sup> Clinically significant lung damage is rare when less than 1000 rads are given within a six-week period, but becomes increasingly more frequent and more severe as the dose is increased above that level. In a series of 248 patients with HD, treated by "mantle irradiation" (see Chapter 50) with a mediastinal dose of at least 4000 rads, 6.4% developed clear evidence of radiation pneumonitis despite careful shielding of lung tissue. However, only one patient died of this.<sup>96</sup> When the lungs themselves were treated for pulmonary HD or for prophylactic purposes with 1500 rads, one third of 69 patients developed pneumonitis; in eight this was severe and in four it was fatal.<sup>96</sup> In typical cases the patient shows no evidence of pulmonary disease for at least two months following completion of therapy and the interval may be



as long as a year. A dry, hacking cough usually is the first symptom. In those with severe cases, dyspnea and all the symptoms and signs of respiratory failure may develop. Chest x ray discloses diffuse, fine, or mottled densities in the affected areas and mediastinal shadows often appear broadened with quite irregular mediastinal-pulmonary borders. Histologic studies indicate an initial interstitial inflammatory reaction with prominent giant-cell infiltration that progresses to fibrosis.

In patients with mild cases, complete resolution of symptoms may take place within two to three months, although permanent "increased markings" may be seen in chest x rays. In other patients, permanent and even fatal degrees of pulmonary insufficiency may result.

No therapy is of proven benefit for radiation pneumonitis, although at least transient symptomatic improvement may result from steroid administration.<sup>98</sup> *Prevention*, by shielding the lung and perhaps by lengthening the time interval in which a total irradiation dose is delivered,<sup>99</sup> is the most effective step that can be taken.

**PERICARDITIS AND PERICARDIAL FIBROSIS.** These complications usually occur only in patients receiving at least 3500 rads to the heart. Clinically apparent pericarditis has been observed in approximately 5% of such patients.<sup>96</sup> From a few months to four years following irradiation, signs and symptoms of acute pericarditis may develop or the acute phase may not be evident, cardiac tamponade due to effusion or to pericardial fibrosis being the first indication of this complication. In other patients, no symptoms are evident, but an enlarging cardiac silhouette noted on chest x ray suggests the onset. In the majority of patients, spontaneous resolution of clinically evident disease occurs, but in some the severity of pericardial fibrosis requires decortication.

**RADIATION HEPATITIS.**<sup>97,99,188</sup> This complication may develop after doses of 2400 rads or more have been delivered to the entire liver. In one series of patients in whom 2450

to 2920 rads were delivered to the liver during the course of abdominal irradiation for ovarian carcinoma, 14 of 65 developed radiation hepatitis which was fatal in 11.<sup>188</sup> Hepatitis is evident within six months of irradiation in most patients, but symptomatic onset may be delayed for as long as three years.<sup>188</sup> Hepatomegaly and hepatic tenderness are common and ascites may develop, but jaundice usually does not occur. Results of liver function tests are quite variable, but serum alkaline phosphatase and bromsulfalein retention are increased in most subjects. The hepatocyte is quite radioresistant; radiation hepatitis appears to be the result of damage to supporting tissue rather than to injury of hepatocytes per se.<sup>99</sup> The incidence and severity of radiation hepatitis in rats is reduced when heparin is given for anticoagulation during radiation.<sup>99</sup> Complete recovery may occur in patients with mild cases or portal hypertension and/or hepatic failure of varying severity may be a permanent and often fatal sequel.

**RADIATION NEPHRITIS.**<sup>96,124</sup> When more than 2000 rads have been delivered to the kidney, radiation nephritis may ensue. Following a latent period of six to twelve months from onset of irradiation, edema, nausea, vomiting, headache, and other symptoms associated with azotemia appear. Urinalysis reveals proteinuria, granular and hyaline casts, and often a fixed specific gravity. The blood urea nitrogen is increased and hypertension may develop. Histologic renal changes include thickening of the capsule, hyaline obliteration of glomerular capillaries, and intertubular fibrosis with tubular atrophy. Progressive renal failure may lead to death. In some patients the condition may become stabilized with continuing, but nonprogressive, renal impairment, and a clinical course similar to that observed in chronic glomerulonephritis may ensue.

**OTHER TOXIC EFFECTS.**<sup>96</sup> The gonads are injured to some degree by almost any dose of irradiation.<sup>96</sup> Spermatogenesis and ovulation are inhibited, transiently by small doses, but permanently by large doses. Approxi-

mately 13% of patients whose *thyroid gland* has been irradiated in conjunction with "mantle" therapy for HD (Chapter 50) have developed hypothyroidism.<sup>96</sup> The growth of the *skeletal system* is affected in children. Transverse myelitis is a rare but serious complication of large-dose irradiation to the *spinal cord*. Other toxic effects of radiotherapy include osteitis, cystitis, and "pulseless disease" presumably due to arteritis.<sup>96</sup>

### The Alkylating Agents

The prototype of the alkylating agents, mechlorethamine (nitrogen mustard, HN2), was the first chemotherapeutic agent of proven benefit against human tumors. It was developed as a result of chemical warfare experimentation during World War II and its clinical usefulness was soon demonstrated.<sup>72,193</sup>

The spectrum of antitumor activity of subsequently synthesized alkylating agents such as chlorambucil, busulfan, cyclophosphamide, and melphalan is similar to that of HN2, but differs to the degree that each has found a useful place in antitumor therapy.<sup>168</sup> Although all of the four drugs just mentioned have the distinct advantage over HN2 of effectiveness when given orally, HN2 continues to have a place in current chemotherapy.

### Mechanism of Action

Alkylating agents, such as HN2, ionize within cells, forming highly reactive intermediate compounds (free radicals) that react with and damage a variety of normal cellular constituents.

The range of their effects upon cells is so broad as to negate exact definition of their primary effect.<sup>189</sup> The  $\beta$  chloroethyl groups of HN2 ionize to become positively charged ethyleniminium derivatives. A major mechanism of the cytotoxicity of HN2 is thought to be the combination of these derivatives with negatively charged guanine moieties of DNA. It seems likely that these drugs are transported into cells by masquerading as

normal compounds for which physiologic carrier systems exist. For example, there is evidence that HN2 gains access to cells via the normal choline transport system.<sup>70</sup>

The similarity of the clinical spectrum of activity of various alkylating agents to that of ionizing irradiation has led to the description of their effects as being *radiomimetic*. Yet, it is clear that the chemical changes induced in cells by alkylating agents differ significantly from those induced by irradiation.<sup>1</sup> A final common pathway of cellular damage may exist, but it is initiated differently by x ray and by various alkylating agents.<sup>1</sup>

The mechanism of resistance to HN2 and to other alkylating agents is unknown.

### Nitrogen Mustard<sup>122,123,153,197</sup>

Mechlorethamine (nitrogen mustard, HN2) has the chemical formula:



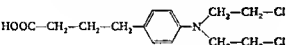
The usual dose is 0.4 mg/kg, given intravenously as a single dose, or 0.2 to 0.3 mg/kg given on each of two successive days. The dose may be repeated every four to six weeks, depending on the rate of recovery of the bone marrow. Once HN2 is solubilized for injection, rapid *in vitro* degradation of the drug begins. Consequently, it must not be placed in solution until the physician is ready to administer it. Since it is a severe vesicant, great care must be taken to avoid injection of any of the drug into cutaneous or subcutaneous tissues. An intravenous infusion of saline or glucose solution should be established through a relatively large-bore needle (18 gauge or larger) and HN2 should be injected through the tubing through which the saline or glucose infusion is flowing freely.

The toxicity of HN2 is dose related and consists of acute gastrointestinal upset and bone marrow depression as well as the previously described vesicant effect. Nausea and vomiting may begin within a few minutes of injection and, while vomiting usually subsides within six hours, nausea and anorexia may persist for a day or two. Both cycling

and  $G_0$  cells that are capable of proliferation are affected by HN2. The nadir of neutropenia and thrombocytopenia usually occurs within two weeks after a dose of HN2 and blood values are back to normal within four weeks in most patients.

**Chlorambucil (Leukeran,  
CB. 1348)**<sup>122,123,153,197</sup>

The chemical formula of chlorambucil is:

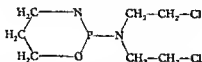


The usual dose is 0.1 to 0.2 mg/kg given orally daily. The reliable oral absorption of chlorambucil and its relatively prolonged plasma half-life as compared to that of HN2 are thought to be due to the substitution of electrophilic groups on the nitrogen atom.<sup>1</sup>

Toxicity is primarily hematologic and in that respect chlorambucil is quite similar to HN2. Nausea, vomiting, and anorexia are unusual. Pulmonary fibrosis, similar to that caused by busulfan, has been mentioned as a rare toxic effect and the possibility of hepatotoxicity has been raised.<sup>3,107</sup>

**Cyclophosphamide (Cytosan,  
CTX)**<sup>122,123,153,197</sup>

Cyclophosphamide's chemical formula is:



Oral or intravenous routes may be used for its administration. Since CTX is not locally irritating, in solution it may be directly and rapidly injected into a vein. The parent compound is not cytotoxic, but becomes so when converted to a variety of intermediates in the liver. When CTX is used in long-term oral therapy, 2 mg/kg, given once a day, is the usual tolerated dose. Larger doses may be given initially for short periods or large-dose intermittent therapy can be used. Thirty to 50 mg/kg can be administered as a single intravenous injection, repeated every three to

four weeks. This regimen may be superior to daily oral therapy in multiple myeloma (Chapter 52), but has not been tested extensively.

The toxicity of CTX is similar to that of HN2 in respect to the bone marrow, but there is suggestive evidence that megakaryocytes are affected less than erythrocyte and granulocyte precursors. Anorexia, nausea, vomiting, stomatitis, and diarrhea are unusual, but may occur. Severe hemorrhagic cystitis that may be fatal occurs and dehydration seems to contribute to the development of hemorrhagic cystitis. Alopecia is common, the nail beds may be affected, and the nails exhibit transverse ridging. Hyperpigmentation of the skin may occur.

**Busulfan (Myleran)**<sup>122,123,153,197</sup>

Busulfan has the following chemical formula:



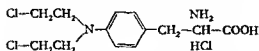
It is given orally, 4 to 6 mg/day in adults, until a desired effect is achieved or toxicity supervenes. Although it is active against a variety of tumors, it is employed almost exclusively in CML (Chapter 48).

Hematologic toxicity, while similar to that caused by HN2, is often of longer duration than that of HN2. This may be explained by the marked and somewhat selective effect of busulfan on pluripotent hematopoietic stem cells observed in mice.<sup>23</sup> Hyperpigmentation of the skin is common and is dose related. Anorexia, nausea, and vomiting are unusual. A variety of rare, but severe and poorly understood, complications attend the use of busulfan. In general, these have been associated with continuous administration of small doses for months or years. "Busulfan lung"<sup>230,219,243,193a</sup> is associated with cough and progressive dyspnea with respiratory insufficiency eventually leading to death. Lung biopsy studies have suggested that the initiating factor is intra-alveolar exudation of fibrin with subsequent organization and fibrosis. A syndrome with features similar to those of Addison's disease has been reported in which adrenal function was normal.<sup>110</sup>

Addison's disease due to ACTH deficiency has also been reported.<sup>173</sup> Amenorrhea may occur. Other reported toxic effects of the long-term use of busulfan include posterior subcapsular cataracts,<sup>146</sup> erythema multiforme,<sup>50</sup> and porphyria cutanea tarda.<sup>109</sup>

**Melphalan (Phenylalanine Mustard, Alkeran, L-Sarcolysin)**<sup>122,123,153,197</sup>

The chemical formula of melphalan is:



Melphalan is given by mouth, either in a dose of 0.05 to 0.1 mg/kg/day as long-term therapy or as a large, intermittent dose of 0.25 mg/kg/day for four days every six weeks (Chapter 52). This L-phenylalanine derivative would be expected to be more active against protein synthetic processes than most other alkylating agents and this may explain its superiority in multiple myeloma.

Toxicity of melphalan seems limited to HN2-like marrow effects and moderately frequent, dose-related anorexia that may be associated with nausea and vomiting.

### Other Alkylating Agents

A variety of other alkylating agents have been synthesized, but have had no apparent advantage over the five widely used drugs that have just been discussed. Thio-TEPA was in widespread clinical use some years ago and was the first orally administered alkylating agent. Its dose-related hematologic toxicity seemed to be more erratic and unpredictable than that of subsequently synthesized alkylating agents and it is now used rarely in the treatment of patients with hematologic neoplasms. One possible current use for the drug may be in the local treatment of malignant effusions (see Chapter 54).

### Antimetabolites

Antimetabolites affect cells by interfering with specific enzymatic conversion of essential metabolites. All useful agents employed

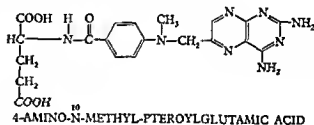
thus far (folate antagonists, 6-MP and related drugs, cytosine arabinoside, and hydroxyurea) affect DNA synthesis and are cycle-active agents.

### Antifolic Acid Compounds

Antifolic acid compounds, first tested clinically in 1947, provided the first useful therapy for AL. Aminopterin was the first agent used, but has been supplanted by methotrexate.

**Methotrexate (Aminopterin, MTX)**<sup>14,87,122,123,153,197</sup>

The chemical formula of MTX is:



The drug can be given by mouth, intravenously, or subcutaneously and is also used for intrathecal therapy (see Chapter 54). A wide variety of dosage schedules have been employed. Some appreciation of the pharmacology of MTX is essential in understanding the rationale of the various regimens and their toxicity. Thus, the time that an effective plasma level is maintained is more important than is the peak plasma level achieved. The drug is filtered and excreted by the kidney in direct proportion to its concentration in plasma and it must be used with extreme caution, if at all, in patients with significant impairment of renal function. Its absorption after subcutaneous injection is faster than after oral administration and, consequently, the effective plasma level may be maintained for a shorter time after subcutaneous than after oral therapy. After intrathecal (i t) injection the drug is released very slowly to the blood. Thus, a single dose of 15 mg given i t may induce hematologic toxicity; 15 mg given orally or subcutaneously will have progressively less effect, while the same dose

given rapidly intravenously may have no detectable effect.

The mechanism of action of MTX<sup>14,87,89</sup> is through competitive inhibition of dihydrofolate reductase, the enzyme that catalyzes the conversion of folic acid to tetrahydrofolic acid (THF) and structurally related derivatives. In the presence of inadequate amounts of THF, DNA synthesis is inhibited and cells become megaloblastic, or, if severely depleted, may die.<sup>57</sup> For practical purposes, MTX therapy is analogous to the induction of a very severe deficiency of folic acid. Folic acid, as such, does not inhibit the effect of MTX, but the coenzyme, citrovorum factor, formed by the acceptance by THF of a single carbon fragment, does. Thus, administration of citrovorum factor at the time of or shortly after MTX administration reduces the effects of MTX. However, there is little evidence that citrovorum factor, given after MTX has been cleared from the blood, will reverse its toxicity.

Resistance to the effects of MTX develops because tumor cells can acquire the ability to synthesize abnormally large amounts of dihydrofolate reductase, thereby degrading MTX and rendering maximally tolerated doses for normal cells ineffective in killing tumor cells.<sup>14,87,89</sup> However, in closed spaces such as the CSF, a concentration of MTX sufficient to overcome the ability of the resistant cells to synthesize the reductase enzyme can be achieved (Chapter 54).

Hematologic toxicity is dose related, but is rapidly reversed by discontinuing use of the drug. Macrocytosis and megaloblastic changes in the marrow usually are not considered indications for interrupting therapy unless accompanied by progressive anemia. Growth of gastrointestinal epithelial cells is markedly affected by MTX with resulting mouth ulcers and diarrhea. Persistent therapy with MTX in patients with diarrhea may lead to hemorrhagic enteritis and perforation of the bowel. Thus, diarrhea and/or mouth ulcers are indications for interruption of therapy. Hepatic dysfunction, as evidenced by abnormal results in liver function tests, is fairly common during MTX therapy.<sup>42,62,92</sup> In a small percentage of patients

receiving long-term therapy, hepatic fibrosis with hepatomegaly and portal hypertension may develop and may prove fatal. Abnormal findings in liver function tests may not be indications for interrupting MTX therapy, but enlargement of the liver is. If therapy is stopped before hepatic fibrosis has become extensive, the fibrotic process usually does not progress further, but it is not reversed. We are familiar with one patient with ALL, currently in her fifteenth year of remission, who has had nonprogressive hepatomegaly with fibrosis and splenomegaly for 13 years after MTX therapy was discontinued. An acute, severe pulmonary syndrome characterized by fever, cough, dyspnea, cyanosis, and diffuse bilateral pulmonary infiltration, which resembled an allergic granulomatous reaction, has been observed during MTX therapy<sup>39</sup> and may prove fatal.<sup>149</sup> Alopecia is common, but dermatitis and nail changes are unusual. Osteoporosis, severe enough to produce pathologic fractures, has developed in children on long-term maintenance therapy with MTX.

Trophoblastic tissue is sensitive to MTX to such an extent that the drug is useful in the treatment of trophoblastic tumors as well as in inducing abortion.<sup>169</sup> MTX is exceedingly teratogenic in man if given in doses too small to induce abortion during the first trimester of pregnancy<sup>132</sup> (Chapter 54).

#### 6-Mercaptopurine (Purinethol, 6-MP)<sup>78, 79, 122, 123, 153, 197</sup>

The chemical formula of 6-mercaptopurine is:



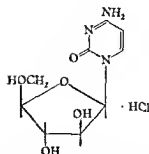
A single, daily, oral dose of 2.5 mg/kg is the standard form of administration, but other forms such as intermittent large doses<sup>63</sup> have not been evaluated extensively. Other purine analogs such as 6-chloropurine,<sup>192</sup> thioguanine, and various derivatives of 6-MP such as 6-MP riboside<sup>50</sup> are similar to 6-MP in antitumor effects and in toxicity.

6-MP reacts with 5-phosphoribosyl-1-pyrophosphate, producing 6-MP ribonucleotide, the active antitumor compound. This reaction is catalyzed by a pyrophosphorylase and one mechanism of resistance to 6-MP may be the emergence of tumor cells containing little or no such enzyme, although this is not always the case.<sup>80</sup> The antitumor effect is exerted primarily by inhibiting purine synthesis and, consequently, DNA synthesis. This may be accomplished by inhibition of conversion of inosinic acid to adenylic and guanylic acid, suppressing *de novo* purine biosynthesis, perhaps by feedback inhibition related to the formation of ribosylamine 5-phosphate from glutamine and 5-phosphoribosyl-1-pyrophosphate, as well as through other effects on purine synthesis. In the belief that the effects of 6-MP are enhanced by allopurinol when the latter is used to prevent hyperuricemia (Chapter 54), it has been the practice to reduce the dosage of 6-MP in such circumstances. However, this recommendation has been challenged.<sup>175a</sup>

Hematologic toxicity of 6-MP is reversed rapidly when use of the drug is discontinued. Anorexia, nausea, and vomiting are fairly common and are dose related, but stomatitis, diarrhea, and severe gastrointestinal toxicity are rare. Jaundice, seemingly on an idiosyncratic rather than on a dose-related basis, is fairly frequent.<sup>38,55</sup> The jaundice is cholestatic in type with bile stasis and some degree of necrosis evident on liver biopsy. The onset of such jaundice generally is considered a contraindication to further 6-MP therapy, but reports of disappearance of jaundice despite continuation of 6-MP therapy have been published.<sup>22</sup> Whether the jaundice was, in fact, due to 6-MP cannot be determined with certainty. Fever has been reported as a complication of 6-MP therapy.<sup>154</sup> As with any drug that has some selective effect on DNA synthesis, megaloblastosis may be induced.

**Cytosine Arabinoside**  
(1-β-D-Arabinofuranosyl  
Cytosine, Cytosar, Ara-C)<sup>120</sup>

Ara-C has the following chemical formula:

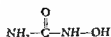


This synthetic, cycle-dependent nucleoside is given intravenously or subcutaneously, as well as intrathecally (Chapter 54). Effective doses are quite similar irrespective of the route of administration.<sup>64</sup> There is no established optimal dosage schedule (see Chapter 47). Ara-C is converted to active phosphorylated derivatives intracellularly and these directly and specifically inhibit DNA synthesis. The exact mechanism of action has not been determined, but the action reflects inhibition of DNA polymerase, at least in part.

Hematologic toxicity is dose related and recovery is prompt after therapy with the drug has been stopped. Megakaryocytes seem exceptionally sensitive to Ara-C and severe thrombocytopenia is often induced. A rebound thrombocytosis may follow cessation of therapy. Anorexia, nausea, and vomiting are common. The mechanism of production of gastric toxicity is unclear, but symptoms may appear within a few minutes after a dose of Ara-C has been given and vomiting rarely persists for more than four to six hours. Diarrhea and oral ulceration are unusual, but may occur. Hepatitis has been reported.

**Hydroxyurea (HU)<sup>34</sup>**

Hydroxyurea's chemical formula is:



This compound has been known for many years, but its antitumor activity was not recognized until the late 1960's. Although optimal dosage schedules have not been developed, HU may be given as an oral dose of 80 mg/kg, daily for three days and that dose may be repeated at six-week intervals, provided toxicity has subsided.

Hydroxyurea inhibits DNA synthesis by

inhibiting ribonucleoside diphosphate reductase and perhaps by other mechanisms as well.

In addition to hematopoietic toxicity, nausea, vomiting, and diarrhea are common, but are usually mild and stomatitis is unusual. Alopecia may occur and rashes, possibly of idiosyncratic nature, have been reported.

### Stathmokinetic Agents

Stathmokinetic agents selectively affect cellular microtubules<sup>126</sup> and thus interrupt formation of mitotic spindles, producing arrest of mitosis in metaphase. Death of the cell usually follows. Thus, these are highly specific, cycle-active antimitotics. However, other effects such as inhibition of DNA-dependent RNA polymerase may also occur.<sup>126</sup>

#### *Vinblastine (Velban, VLB)*

Vinblastine, an alkyloid derived from the periwinkle plant, is given intravenously, initially in a single dose of 0.1 to 0.15 mg/kg. This dose is repeated every seven to ten days and is increased slowly to as much as 0.3 mg/kg if not prevented by severe toxicity.

Hematologic toxicity is transient and dose dependent. Nausea, vomiting, and anorexia are fairly common, as is stomatitis. Either diarrhea or constipation may occur. Neurologic complications like those associated with vincristine are uncommon, but may occur, as may alopecia.<sup>27</sup>

#### *Vincristine (Oncovin, VCR)<sup>49</sup>*

Vincristine is extracted from the same plant as VLB and is very closely related in chemical structure, but there are significant differences in the spectra of antitumor activity (see Table 55-2) and type of toxicity of the two drugs.

VCR is given intravenously in a once weekly dose of 1.0 to 1.5 mg/m<sup>2</sup>. The total dose/week should not exceed 2 mg. Differences in the mechanism of action of VCR and VLB have not been elucidated.

Toxicity is primarily neurologic (Table 55-5).<sup>150</sup> Loss of deep tendon reflexes in the legs and mild paresthesias, usually involving the feet and hands, may occur after the first dose and are present in most patients by the third dose. Many physicians do not consider loss of reflexes in the legs and mild paresthesias as contraindications to further therapy, and in one series of 100 patients no serious toxicity was noted with a total cumulative dose of less than 7 mg.<sup>49</sup> However, patients may develop severe paraparesis when another dose is given in the face of minimal neurologic toxicity and, unless there are compelling reasons to give additional VCR, it usually is better not to. Likewise, severe complications, such as bladder atony<sup>73</sup> have followed minimal doses without other premonitory neurologic changes. Certainly the onset of severe paresthesias, any motor weakness in the extremities, hoarseness, ptosis, double vision, or severe constipation should be indications for stopping VCR therapy. Improvement occurs in neurologic lesions when therapy is stopped, but recovery may require months and permanent damage may remain if toxicity is severe. Alopecia is common, but hematologic toxicity is unusual. Only in an occasional patient is severe bone marrow aplasia produced.<sup>150</sup>

### Other Stathmokinetic Agents

*Colchicine* has a stathmokinetic effect, but only in vivo at dosages precluded by nausea and vomiting. A colchicine-like agent, *demicolcine*, was shown to be effective in CML,<sup>117</sup> but since it offers no advantage over busulfan, it is no longer available.

### Antibiotics

Antibiotics are antitumor compounds derived from living organisms.

#### *Daunomycin (Daunorubicin, DNR)*

Daunomycin was isolated from *Streptomyces peucetius*.<sup>77</sup> Optimal dosage schedules are not available, but this antibiotic is usually

**Table 55-5. Comparison of Drug-Related Neurologic Manifestations in Leukemic Patients Receiving Chemotherapy with and without Vincristine\***

<i>Clinical Manifestations</i>	<i>Combination Chemotherapy Including Vincristine (50 Patients)</i>	<i>Combination Chemotherapy without Vincristine (20 Patients)</i>
	<i>Number of Cases</i>	<i>Number of Cases</i>
Depression or loss of Achilles tendon reflex	50	0
Depression or loss of other deep tendon reflexes	24	0
Motor weakness (associated with neurologic dysfunction)	17	0
Slapping broad-based gait	11	0
Flaccid paralysis, lower extremities	2	0
Muscle pain and tenderness	4	3
Muscle atrophy	5	3
Paresthesias		
(combination chemotherapy only)	19	0
(chemotherapy and anti-biotics)	4	2
Discrete sensory deficits	0	0
Diplopia,		
other visual disturbances	6	3
Ophthalmoplegia (VI)	3	0
Ptosis (III)	5	0
Facial palsy (VII)	2	1
Paroxysmal jaw pain (V)	2	0
Mild abdominal pain	23	4
Moderate to severe abdominal pain, ileus	6	1
Difficulty in initiating micturition	2	0

\*From Sandler et al.<sup>150</sup> courtesy of the authors and Neurology

employed in brief "bursts" such as 2 mg/kg/day, given intravenously for three days.

A cytoplasmic enzyme, daunorubicin reductase, converts daunomycin to an active compound, daunorubinol, in the presence of NADH.<sup>90</sup> There is evidence to suggest that the myeloblasts of patients with AML who respond favorably to DNR contain high levels of this enzyme.<sup>76,90</sup> Synthesis of RNA and DNA is inhibited and there is specific interference with the G<sub>2</sub> phase of the cell cycle as well.<sup>167</sup>

Toxicity of DNR is severe. Marrow apla-

sia, seemingly of irreversible nature, may be produced, suggesting that DNR may not be a cycle-active agent exclusively. Cardiac toxicity, a severe cardiomyopathy which may be fatal, is partially dose related. It is uncommon in children unless a cumulative total dose of 900 mg/m<sup>2</sup> has been given, but has occurred in older patients with smaller doses.<sup>167</sup> Thus, there may be some hazard of cardiac toxicity with any biologically active dose of the drug. Other side effects include nausea, vomiting, and diarrhea; alopecia is common.

Drugs similar to daunomycin, such as the hydroxy derivative of daunomycin, *adria-*



mycin,<sup>34</sup> or a semi-synthetic methylated derivative,<sup>13</sup> are also active antitumor antibiotics. Whether their antitumor effect and their therapeutic/toxic ratio will lead to their replacing DNR in clinical use remains to be determined.

### *Bleomycin*<sup>17a,194,197</sup>

Bleomycin is the generic name for a group of related sulfur-containing glycopeptide antibiotics derived from *Streptomyces verticillatus*.<sup>174</sup> It is given intravenously, but optimal dosage schedules have not been defined; tolerable dose levels lie between 4 and 15 mg/m<sup>2</sup> administered twice weekly for a total of 12 doses.<sup>142</sup> According to one regimen, 15 mg are given daily for six days and then once weekly. Effects seem largely limited to lymphoid tumors and tumors of the skin.

The mechanism of action has not been elucidated completely, but cells are arrested in G<sub>2</sub>. At higher doses, DNA synthesis is inhibited while RNA and protein syntheses are little affected, and there is evidence for scission in single-stranded DNA.<sup>194,197</sup>

Bleomycin has no evident hematologic toxicity.<sup>23a</sup> If total doses in excess of 100 mg/m<sup>2</sup> are given, serious pulmonary fibrosis may become evident and commonly progresses to fatal pulmonary insufficiency.<sup>15</sup> The skin and skin appendages, such as nail beds, are affected and become inflamed and sclerotic, and alopecia is frequent. Nausea and vomiting may occur immediately after the drug is given and fever and chills are common. On occasion, anaphylactic shock and disseminated intravascular coagulation have been observed.

### *Other Antibiotics*

*Actinomycin D* and *mithramycin* are antibiotics that complex with DNA and inhibit DNA-directed RNA synthesis. They rarely have been used and have not been fully evaluated in the treatment of hematologic malignancies, in part at least because of their severe and varied toxic effects. The use of low doses of mithramycin in the control of hypercalcemia has been discussed (Chapter 52).

### Adrenal Glucocorticosteroids ("Steroids")<sup>37</sup>

The natural or synthetic steroids such as hydrocortisone, prednisone, prednisolone, or dexamethasone and ACTH all have the same spectrum of antitumor activity insofar as has been determined (see Table 47-5, page 1490, for comparison of ACTH, hydrocortisone, and prednisone in ALL). These agents are useful in the management of certain lymphoid neoplasms (Table 55-2).

The specific mechanism of the lympholytic effect of steroids is unknown.<sup>37</sup> Nonproliferating cells as well as those in cycle are affected and protein synthesis and mitosis are inhibited.<sup>56,100,139,180</sup> Lymphoid cells may have specific membrane-binding sites which may be used by steroids, such binding leading to cell death.<sup>10,118a</sup> Low levels of transcortin may enhance the lympholytic effect of steroids.<sup>182</sup> In patients with ALL who were no longer sensitive to steroids, binding sites were not demonstrable in lymphoblasts.<sup>10,118a</sup>

The optimum dosage of steroids has been fairly well delineated in ALL (Chapter 47). Higher doses are no better than a dose of 60 mg of prednisone per day in adults. There is no evidence that very large doses are advantageous in any of the other hematologic neoplasms. Thus, 1 mg/kg/day of prednisone given orally, or equivalent doses of other steroids, seems reasonable as initial therapy in adults, as does 2 mg/kg in children. However, although the lympholytic effect of steroids is rapid, it is transient. Alternate-day therapy, which reduces toxic effects, is not particularly effective in lymphoid neoplasms.<sup>125</sup> Maximum effect is achieved in four to six weeks in ALL (Chapter 47), CLL (Chapter 49), HD (Chapter 50), and NHL (Chapter 51). If the therapy is stopped within six weeks, repeated responses at later phases of disease may be obtained. Maintenance therapy with any dosage of steroids is clearly contraindicated in patients with ALL (Chapter 47) and is of no proven benefit as antitumor therapy in patients with any of the other hematologic malignancies.

The toxic effects of pharmacologic doses of steroids are so well known, so frequent,

and so varied that a comprehensive discussion is unnecessary here. They are listed in Table 13-6 (page 555). The use of steroids in short bursts of therapy, the best means of utilizing their lympholytic effect, does not produce many of the long-term ill effects associated with long-continued therapy, such as severe osteoporosis. However, infection, peptic ulceration, and diabetes are encountered with some frequency.

Infections with bacteria, fungi, and certain viruses, such as the herpes viruses, are more severe, if not more frequent, in patients receiving steroids<sup>116</sup> than in patients not given steroids. The reasons for this are not entirely clear. Steroids stabilize lysosomes and thus may inhibit killing of phagocytized organisms. Reduced reticuloendothelial clearance of injected particles has been reported in animals given steroids. Hydrocortisone and prednisone reduce the migration of phagocytes into inflammatory exudates, an effect that seems to provide a satisfactory explanation for the occurrence of severe infections during such therapy.<sup>17</sup> However, it has been reported that dexamethasone does not have such an effect and, in fact, increases the number of phagocytes entering an induced exudate in man.<sup>158</sup> Antibiotics used in prophylaxis against infection in steroid-treated patients have been ineffective, if not harmful.<sup>116</sup>

Other toxic effects encountered occasionally include panniculitis,<sup>94</sup> myopathy,<sup>115</sup> avascular necrosis of the femoral head,<sup>82</sup> and fatty infiltration of the liver and kidney.<sup>159</sup>

### Miscellaneous Agents

#### *L*-Asparaginase<sup>32,140</sup>

Inhibition of growth of murine neoplasms by guinea pig serum<sup>97</sup> was found to be the result of the presence of an enzyme, *L*-asparaginase,<sup>28</sup> and clinical trials of this enzyme, harvested from *E. coli*, were initiated. Certain human tumor cells apparently require exogenous asparagine for growth, although asparagine is generally considered a nonessential amino acid. *L*-Asparaginase

reduces blood asparagine to undetectable levels, thereby depriving the tumor cell of this amino acid.

*In vitro* susceptibility of leukemic cells to *L*-asparaginase unfortunately is not particularly useful in predicting *in vivo* effect.<sup>86,175</sup> This may be due to the ability of certain tumor cells to enhance asparagine synthesis by increasing their level of asparagine synthetase activity in response to exogenous asparagine depletion.<sup>86</sup>

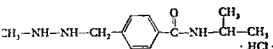
*L*-Asparaginase is given intravenously, but the optimal dose has not been defined. In initial trials in patients with ALL, very large doses, in excess of the dose needed to depress blood asparagine to unmeasurable levels, were used.<sup>32</sup> Doses as small as 1000 international units (iu) once or twice per week may be as effective as larger doses.<sup>84a,140</sup>

The true toxicity of *L*-asparaginase is difficult to define since this enzyme is not chemically pure. Many of the reported side effects may be due to contaminants, such as foreign protein and bacterial endotoxin. Certainly the occasional occurrence of anaphylactic shock suggests that *L*-asparaginase contains strong, foreign antigens. Reported side effects include severe hepatitis and pancreatitis; anorexia, nausea, vomiting, and diarrhea; fever; weight loss, lethargy, somnolence, and confusion; disseminated intravascular coagulation; hypo- and hyperlipidemia; hypocalcemia and azotemia. No effects are noted on normal bone marrow cells in most patients. However, in an occasional patient, complete aplasia is produced.<sup>113</sup> We have seen two such patients and in both rapid marrow recovery occurred.

Toxicity may be reduced by giving relatively small doses (1000 iu/wk rather than 10,000 iu/wk), but even quite small doses have been associated with very severe toxicity.<sup>140</sup> Of 105 children with ALL<sup>113</sup> who were given 200 iu/kg/day, 45 developed serious toxicity: anaphylaxis occurred in seven, pancreatitis in four (all died), hyperglycemia in four (one died), hepatotoxicity in 14 (two died), marrow aplasia in two (one died), atopic rash in four, CNS signs in nine, and hypertension in one, with a total fatality rate of almost 8%.

*Procarbazine (Natulan<sup>®</sup>, PC)<sup>33,34</sup>*

Procarbazine has the chemical formula:



This methylhydrazine derivative is given in an oral dose of 50 to 150 mg/m<sup>2</sup>/day with appropriate adjustment of dose when toxicity occurs.

PC may act in part as an alkylating agent, but additional mechanisms of action exist, as suggested by the fact that cross resistance with other alkylating agents is not evident clinically. It inhibits synthesis of DNA and RNA as well as protein, but the mechanism of action is not completely understood.<sup>33</sup>

Toxic effects, in addition to marrow depression, include anorexia and, less commonly, nausea and vomiting. Central nervous system depression and peripheral neuropathy may occur, particularly in the elderly, but are largely avoided if daily doses do not exceed 300 mg. The drug is an effective monamine oxidase inhibitor, an action that must be kept in mind when prescribing other drugs during procarbazine therapy.

*Nitrosoureas<sup>199</sup>*

Nitrosoureas are a relatively new class of compounds of which 1,3 bis (2-chloroethyl)-1-nitrosourea (BCNU) is the parent compound.<sup>34,198</sup> BCNU is given intravenously in single doses ranging from 100 to 250 mg/m<sup>2</sup>.<sup>198</sup> It is dissolved in absolute methanol, diluted in saline solution, and infused in 10 to 30 minutes. Because of delayed and unpredictable hematologic toxicity the dose should not be repeated for four to six weeks.

Purine incorporation into both DNA and RNA is inhibited by BCNU, perhaps because of inhibition of de novo purine synthesis. Similar effects are observed with alkylating agents (page 1721). However, since BCNU has been of benefit in patients with HD who were resistant to the antitumor effects of alkylating agents, the mechanisms of action of BCNU and alkylating agents probably differ. It does

seem clear that BCNU affects noncycling as well as cycling cells.

Hematologic toxicity is the most serious side effect. This is often delayed and of unpredictable degree. The nadir of neutrophil and platelet depression is not reached in the average patient until 30 days after a single dose has been given.<sup>198</sup> Fatal aplasia is a hazard any time the drug is used.

Within two hours following injection, flushing of the skin and conjunctivae is often noted, effects which clear rapidly. Nausea and vomiting may occur acutely and esophagitis, anorexia, dysphagia, and diarrhea have been noted occasionally. BCNU is a mild vesicant if infused into subcutaneous tissue. Severe hepatic and renal toxicity is induced in animals, but, except for mild, transient abnormalities denoted by liver function tests and elevation of blood urea nitrogen, hepatic and renal toxicities have not been noted in man.

Nitrosoureas closely related to BCNU, such as CCNU and methyl CCNU, are more lipid soluble than BCNU and have two pharmacologic advantages as compared to the parent compound. Their lipid solubility allows them to be used as oral rather than intravenous agents and they cross the blood-brain barrier more readily than does BCNU. Their activity against hematologic neoplasms and the toxicity of these agents, as compared to BCNU, remain to be determined.

*Other Agents*

*Dibromomanitol* is effective in CML patients at a dose of 250 mg/day administered orally, but a comparison with busulfan is not yet available nor has this drug received extensive trials in patients with other hematologic tumors. *Methy-GAG* (methylglyoxal-bis-guanylhydrazone) is active in AML, but its hematologic, gastrointestinal and cutaneous toxicity are so severe that trials have been limited. *Mitomycin-C* has proven antitumor effects in HD, NHL, and CML, but the antitumor dose and the dose causing bone marrow toxicity are so close that this agent is rarely used. The same objection limits the

use of *streptonigrin*, an agent with significant effects in NHL and HD. *Rifamycin* antibiotics, while of no proven benefit in human tumors, are interesting in that they constitute a new class of agents that inhibit RNA-dependent DNA polymerase (Chapter 46). Certain derivatives of the parent compound, rifampicin, have significant inhibitory effects on viral RNA-dependent DNA polymerase and have a greater effect on the DNA polymerase of leukemic cells than on that derived from normal cells.<sup>165,195</sup> The synthetic, double-stranded RNA, *poly 1:C*, induces production of interferon, is a moderately effective antiviral agent, and has proven effects against certain virus-induced murine neoplastic diseases.<sup>197</sup> It represents a new class of agent, not yet tested in human neoplasms. Other agents of possible value, but as yet not fully tested in human tumors, include *streptozotocin*, an antibiotic that is chemically related to the nitrosoureas; *N-demethylepipodophyllotoxin thenylidene gluconide*, a stathmokinetic agent; and *5-azacytidine*<sup>96a</sup> and *guanazole*, drugs related to cytosine arabinoside.<sup>29</sup>

## Experimental Forms of Therapy

A variety of forms of therapy other than those discussed above have been or are being tested in the hematologic neoplasms, particularly in acute leukemia.

### Bone Marrow Transplantation

Successful transplantation of human bone marrow is possible between identical twins and also between non-twin siblings in whom the four major histocompatibility loci (HLA loci) (Chapter 12) are identical.<sup>44,59</sup> However, only between genetically identical siblings (twins) can transplantation be done with some degree of impunity. Identity of the HLA loci, while crucial to successful transplantation, does not insure tissue compatibility, which obviously depends on other loci as well.

The recipient is treated with potentially lethal doses of whole-body irradiation or cyclo-

phosphamide prior to transplantation.<sup>152,172</sup> This provides immunosuppression and also markedly reduces the total mass of leukemic cells. Large volumes of normal marrow are aspirated, filtered to remove clumps, and injected intravenously. If graft-versus-host disease develops,<sup>43,74,108,164a</sup> treatment is given with steroids and MTX, but this may be of no avail and the patient may die with severe liver and skin damage. A period of profound pancytopenia always follows engraftment before the new marrow grows to a functional size.

Complete remissions of acute leukemia have followed such engraftment, but most have been of short duration with leukemia recurring quickly.<sup>43,75</sup> As discussed in Chapter 46, leukemia has occurred in the engrafted marrow in at least two instances. In terms of clinically useful therapy, marrow transplantation may have a greater role in treating patients with aplastic anemia or immune deficiency than those with leukemia.<sup>170</sup>

*Autologous marrow* can be obtained before therapy, stored, and reinfused, in the hope of permitting administration of larger doses of chemotherapy or irradiation to patients with diseases such as HD, but the benefit of this procedure is questionable.<sup>106</sup>

*Extracorporeal irradiation* of the blood has had some salutary effects in the chronic leukemias, but has not induced remissions in acute leukemia.<sup>4,36</sup> The spleen has been irradiated selectively in patients with lymphoma by passing a catheter into the splenic artery via the aorta and injecting ceramic or plastic microspheres in which <sup>90</sup>yttrium has been incorporated.<sup>8</sup>

### Immunotherapy

The possibility of modifying the growth of human neoplasms by administering specific antibodies or by causing the patient to mount an immune reaction against his own tumor has been an attractive hypothesis, intermittently pursued at the clinical level since the turn of the century.<sup>60,135</sup> The recent and rapid growth of our knowledge concerning immunity in general (Chapter 7) and of

tumor immunity in particular<sup>60,127,166</sup> has provided a sound base for undertaking clinical trials of immunotherapy. However, proof that useful immunotherapy of hematologic malignancies can be accomplished in man is lacking as yet, although it has been shown to be useful in a number of animal models.<sup>60,130,147</sup>

A number of observations suggest that immunotherapy may be feasible. First, there is evidence that most human tumors,<sup>136</sup> including leukemias,<sup>18,134,178</sup> possess a unique cell surface antigen not found on normal cells (Chapter 46). Thus, there is reason to believe that an immune response that would be specific for the tumor and fail to affect normal cells could be mounted. Second, the occurrence of spontaneous remissions in hematologic as well as in other types of malignant disease suggests that the host can somehow gain control of his own malignant condition. While this control could be due to factors other than immune mechanisms, there is excellent evidence that spontaneous resolution of murine neoplasms has an immune basis.<sup>60</sup> Third, there is evidence that an immune response to tumor is mounted by the human host.<sup>5,160</sup> The histologic appearance of the cellular response surrounding tumors or in lymph nodes draining tumors often resembles that noted with delayed hypersensitivity reactions or graft rejections. In vitro lymphocyte-induced tumor cytotoxicity has been demonstrated in some instances. For example, the lymphocytes from patients with ALL in remission are stimulated by exposure to autologous leukemic cells.<sup>66,118,144</sup> Circulating specific antibodies to the host's tumor are often demonstrable, as is blocking factor. Blocking factor, so named because it interferes with cytotoxic antibodies in vitro, probably consists of antigen-antibody complexes. Fourth, there is evidence that tumors may develop more frequently or spread more rapidly in the presence of immune deficiency. As discussed in Chapter 46, lymphomas have developed with greater than expected frequency in patients with congenital or iatrogenic immune deficiency. The prognosis of patients with tumors is poor if cell-mediated

immunity is impaired as compared to the course of those in whom it is intact.<sup>134,166</sup>

Thus, a basis for attempting immunotherapy of human tumors has been developed and in general it would appear that cell-mediated immunity is important in the control of tumors, while humoral immunity could be harmful as well as helpful.<sup>147</sup> The possible harmful nature of humoral immunity stems from the aforementioned blocking factor.<sup>85</sup> The presence of blocking factor may reflect excessive soluble tumor antigen compared to the amount of specific tumor antibody that is present and, indeed, its presence has been correlated with glomerular lesions similar to those seen in experimentally induced "antigen excess" nephritis.<sup>166</sup> Blocking factor reversibly inhibits the in vitro antitumor cytotoxic effect of sensitized lymphocytes. There is also a "deblocking" factor that may simply represent excess antibody, unbound to tumor antigen.<sup>127,134</sup> There is suggestive evidence that blocking factor may play an adverse role in vivo.<sup>83,134,166</sup> It often is demonstrable in the presence of active disease, but disappears after successful excision of tumor.

The methods being tested as approaches to immunotherapy can be divided into three categories: (1) active immunotherapy, (2) passive immunotherapy, and (3) nonspecific immunotherapy.<sup>134</sup>

*Active immunotherapy* consists of attempting to alter tumor antigens so that their antigenicity is enhanced. The most efficient active immunotherapy in animals may consist of intradermal injection of living, autologous tumor cells in insufficient number to produce a tumor.<sup>30,134</sup> This approach has not been utilized in man for fear that viable tumor cells would grow. Instead, cells rendered nonviable by irradiation, mitomycin C, freezing and thawing, or heating have been employed and highly antigenic carrier proteins have been added in an attempt to increase antigenicity in human experiments.<sup>134</sup> Isolation of pure preparations of human tumor antigens for injection has not yet been accomplished.

*Passive immunotherapy* consists of administering antitumor sera or lymphocytes. Antitumor sera, produced in animals against

human tumors, have been of little, if any, benefit.<sup>134</sup> However, as blocking factor, de-blocking factor, and humoral cytotoxic antibody relationships are clarified, antiserum therapy should probably be reevaluated. Lymphocytes from HLA-matched siblings or autologous lymphocytes grown in culture<sup>28,65,134</sup> or stimulated *in vitro* with PHA<sup>12,134</sup> have been infused with some suggestion of clinical benefit.<sup>134</sup> Transplantation of the spleen from a normal person to his identical twin who had lymphocytic leukemia was accomplished, but without appreciable benefit.<sup>135</sup> Studies of patients cross sensitized by injecting each others' tumors and then exchanging lymphocytes<sup>31,133,134</sup> are somewhat difficult to interpret since the sensitized lymphocytes would not survive the HLA barrier for an appreciable length of time. No evident effect was observed in patients with acute leukemia who were infused with lymphocytes from volunteer donors who had been injected with the patients' leukemic cells.<sup>6</sup>

Transfer factor,<sup>114</sup> the factor derived from lymphocytes which is capable of passing information of specific immune response from sensitized to nonsensitized patients (Chapter 7), is being investigated as a possible method of increasing the tumor-bearing host's immunity, as is immune RNA that can be extracted from sensitized, xenogeneic lymphocytes.<sup>47</sup>

*Nonspecific immunotherapy* is based on the thesis that if the host's immune system is stimulated by a nonspecific antigen, immune response to other antigens will be enhanced. Probably it was first used as antitumor therapy by Coley,<sup>138</sup> who observed regression of a tumor after erysipelas; this led him to develop a series of bacterial toxins whose injection led to tumor regression in some patients. Since it is now thought that the cellular immune system is primarily responsible for tumor immunity, rather than the humoral system, which was probably stimulated by Coley's toxins, agents that stimulate the cellular system, such as BCG, are under investigation. When BCG is injected into patients with leukemia or other tumors a

measurable increase in the host's antitumor immune response can be induced.<sup>134</sup> Proof of clinical benefit in such trials is lacking in leukemia patients (Chapter 47), but there is probable benefit in those with other tumors, such as melanoma.<sup>134</sup> "Adoptive" immunotherapy<sup>127</sup> is similar to passive immunotherapy in that lymphocytes from nonimmunized, but allogenic donors are given to patients; some moderate clinical benefit has been reported in patients with ALL<sup>156,178</sup> and in patients with other tumors.<sup>196</sup>

Since, in most animal models, immunotherapy is effective as preventive therapy or in reducing a very small volume of transplanted tumor, many current trials in man are directed toward attempting to prolong drug-induced remissions rather than with the object of reducing demonstrable tumor.<sup>128</sup>

### Other Forms of Therapy

*Exchange transfusion* led to transient remission in some patients with acute leukemia.<sup>15,16,130</sup> A controlled study that compared transfused fresh versus stored blood revealed that fresh, but not stored, blood reduced the blood leukocyte count in patients with leukemia.<sup>185</sup> Limited trials of fresh plasma infusions were of no apparent benefit in acute leukemia,<sup>35,45,79</sup> but no systematic investigation of the factor in fresh, normal blood, which influences leukemia, has been carried out.

Early attempts to treat leukemia as if it were a *deficiency disease* by giving liver, gastric mucosa, pancreas, small intestine, bone marrow, and embryonic rabbits or extracts thereof, were unsuccessful.<sup>35</sup> Thymectomy<sup>16,95</sup> and adrenalectomy<sup>183</sup> have been without apparent effect in ALL and AML, respectively. Transient repeated improvement was reported in a patient with AML given Sendai, Newcastle, and influenza virus,<sup>190</sup> and injection of Langat and Kyasanur forest disease virus led to improvement in a few patients with leukemia and in patients with a variety of cancers.<sup>179</sup> Induction of amino acid imbalance was of no apparent benefit.<sup>2</sup>

## Development of New Agents

The empiric program to develop and screen new anticancer drugs which is sponsored by the Drug Research and Development section of the National Cancer Institute is a primary source for the new drugs that reach the stage of clinical trials.<sup>69</sup> In this program, all agents are tested for effects upon growth of L-1210 leukemia, transplanted into mice. Crude natural products are also routinely tested for their effect on P-388 mouse leukemia. The magnitude of this program is illustrated by the fact that more than 49,000 crude natural plant extracts were tested through 1971. Materials that show activity in the primary screen also are tested for effects on B-16 mouse melanoma, mouse Lewis lung carcinoma, rat Walker carcinoma 256, and in an *in vitro* KB cell culture system. Those that show promise in the primary screens are then evaluated further in the following stepwise fashion: toxicity studies in dog and monkey, clinical trials to determine tolerated doses in man (phase I clinical trials), estimation of therapeutic activity in human cancer (phase II), and, finally, definitive trials designed to eradicate potentially sensitive human tumors (phase III).

In general it can be stated that most agents that have reached clinical trials have given evidence of antitumor activity in man, although the majority have not found widespread clinical use, either because of prohibitive toxicity or because they offered no apparent advantage over existing agents. Animal studies generally have been of value in predicting qualitative toxicity of various agents for man, but some unpredicted toxicity for specific organs has been encountered.<sup>88</sup> Tolerated dose levels in mice, rats, and dogs, in general, have been found to be similar to those in man when calculated on the basis of surface area rather than weight.<sup>88</sup>

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# Part VI

## Pancytopenias and Myelophthasic Conditions



## *Pancytopenia, Aplastic Anemia, and "Pure Red Cell" Aplasia*

Pancytopenia  
Aplastic Anemia  
Acquired Aplastic Anemia  
Constitutional Aplastic Anemia  
Fanconi's Syndrome (Congenital Pancytopenia)  
Aplastic Anemia with Pancreatic Insufficiency  
Pure Red Cell Aplasia  
Acquired Acute Erythropoietic Hypoplasia  
Chronic Acquired Erythropoietic Hypoplasia  
Constitutional Erythroid Hypoplasia

### **Pancytopenia**

#### **Definition**

*Pancytopenia* refers to a reduction in all three formed elements of the blood—the erythrocytes, leukocytes, and platelets. It is not a disease entity; rather it is a triad of findings that may result from a number of disease processes (Table 56-1).

#### **Pathophysiology**

The mechanism by which pancytopenia develops has not been studied extensively. In some of the conditions listed in Table 56-1 there is a marked decrease in hematopoietic cell production in the bone marrow either as

a result of destruction of marrow tissue by toxins (acellular or hypoplastic marrow) or perhaps because of replacement by abnormal or malignant tissue. In such patients, bone marrow biopsy usually provides the diagnosis. In other patients, however, the marrow may be normally cellular or even hypercellular<sup>7,35,44,48,53</sup> and no abnormal cells may be present. The mechanisms leading to pancytopenia in such patients are thought to include: (1) ineffective hematopoiesis with cell death in the marrow; (2) formation of defective cells that are rapidly removed from the circulation; (3) sequestration and/or destruction of cells as a result of antibodies; and (4) trapping of normal cells in a hypertrophied and overactive reticuloendothelial system. Ferrokinetic and red cell kinetic studies have demonstrated active but ineffective erythropoiesis in some patients,<sup>16</sup> and it seems likely that the same mechanisms apply to granulocytes<sup>4</sup> and platelets.<sup>20</sup> However, methods for studying cell kinetics are not generally available in most clinics; consequently, ineffective hematopoiesis is inferred when there is no marrow hypoplasia.

#### **Causes**

The diverse causes of pancytopenia are listed in Table 56-1. Most of the diseases are discussed elsewhere, as indicated in the table;

Table 56-1 Causes of Pancytopenia

- 1 Disorders infiltrating the bone marrow
  - a Aleukemic leukemia (Chapter 47)
  - b Multiple myeloma (Chapter 52)
  - c Metastatic carcinoma (leukoerythroblastic blood picture Chapter 57) lymphoma (Chapters 50, 51)
  - d Myelofibrosis myeloid metaplasia (Chapter 57)
  - e Marble bone disease osteopetrosis (Chapter 57)
- 2 Disorders involving the spleen
  - a Congestive splenomegaly (Chapter 45)
  - b Lymphomas Hodgkin's disease non Hodgkin's lymphoma (Chapters 50 and 51)
  - c Infiltrative disorders Gaucher's disease, Niemann-Pick's disease (Chapter 42), Letterer-Siwe disease (Chapter 42)
  - d Infectious diseases kala-azar miliary tuberculosis syphilis
  - e Primary splenic panhematopenia\* (Chapter 42)
- 3 Vitamin B<sub>12</sub> or folate deficiency—pernicious anemia sprue etc (Chapters 14-15)
- 4 Disseminated lupus erythematosus
- 5 Paroxysmal nocturnal hemoglobinuria (Chapter 29)
- 6 Miscellaneous disorders (with cellular marrow)—overwhelming infection mycobacterial infections (some cases) brucellosis sarcoid pregnancy (some cases) some refractory anemias sideroblastic anemia (rarely) and perhaps drug sensitivity<sup>24</sup>
- 7 Aplastic or hypoplastic anemia
  - a Acquired
    - (1) Chemical and physical agents (Table 56-2)
      - (a) Agents that regularly produce aplasia if dose is sufficient—ionizing radiation, benzene, etc
      - (b) Agents only occasionally associated with hypoplasia—drugs
    - (2) Other causes of aplastic or hypoplastic anemia—certain viral infections (hepatitis, dengue) some mycobacterial infections, pregnancy, Simmond's disease, sclerosis of the thyroid
    - (3) Idiopathic
  - b Familial—Fanconi's constitutional pancytopenia pancreatic deficiency in children

therefore, only a few unusual causes and the aplastic anemias will be considered here.

### Disseminated Lupus Erythematosus<sup>22</sup>

Anemia, thrombocytopenia, or leukopenia is commonly present in patients with dissem-

inated lupus erythematosus.<sup>22,31</sup> Anemia is manifest in most patients during the course of this disease. Thrombocytopenia may be severe and bleeding phenomena are not unusual even as initial complaints.<sup>22</sup> Simultaneous depression of erythrocytes, platelets, and leukocytes is more unusual, being evident perhaps only in 5% of these patients. Moreover, the leukopenia, if present, is mild (rarely less than  $2.0 \times 10^9$  cells/ $1^{23}$ ), and neutrophils usually constitute at least 50% of the cells present. As a rule these patients can respond to infection with an increase in neutrophils and a shift to the left.<sup>34</sup> The bone marrow usually is cellular but may be hypocellular on rare occasions.<sup>22,31</sup> The diagnosis is suggested by symptoms and signs such as arthritic manifestations, skin lesions, and polyserositis, which often precede the appearance of hematologic manifestations.

### Miscellaneous Disorders

Severe overwhelming infection rather than being a direct cause of pancytopenia usually reflects the exhaustion of marrow reserves already depleted, eg, as a result of vitamin deficiency (B<sub>12</sub> or folate), alcoholism, the effect of drugs or chemicals to which the patient may be sensitive, or previous treatment with cytotoxic agents. The marrow in such subjects often is cellular but with few mature neutrophils present, and there is an apparent increase in immature forms, thereby giving the appearance of so-called "maturation arrest"; the bone marrow picture may even simulate that of acute leukemia (see page 1302). Pancytopenia has been reported occasionally in association with tuberculosis<sup>10,50</sup> and other mycobacterial infections such as *M. kansasii*.<sup>25</sup> Clinical features included fever, sweating, weight loss, lymphadenopathy, and splenomegaly. Macrocytosis and severely hypoplastic or aplastic bone marrow containing caseating tubercles were present in some patients while in others the marrow was cellular. Miliary infection involving the spleen and lymph nodes was the usual finding. Occasional apparent cures have followed splenectomy and appropriate antibiotic ther-

apy,<sup>15,50</sup> but more often the presence of tuberculosis represents only the coincidental occurrence of this infection in patients with leukemia or other dyscrasias.<sup>18</sup> Rarely pancytopenia has been reported in association with brucellosis,<sup>33</sup> sarcoid, pregnancy,<sup>305</sup> idiopathic sideroblastic anemia,<sup>29</sup> and refractory anemia, some of which conditions, after a variable period, may evolve into acute myelomonocytic leukemia, erythroleukemia, myelofibrosis, or aplastic anemia.<sup>33</sup>

### "Bicytopenia"

Commonly patients are encountered in whom only two of the formed elements are present in decreased concentration.<sup>2</sup> This may occur in association with most of the disorders listed in Table 56-1, either as a persistent finding or as pancytopenia is developing. Other examples can be found, eg, congenital rubella with thrombocytopenia and hemolytic disease in association with decreased megakaryocytes in an otherwise cellular marrow<sup>40</sup>; severe refractory anemia and leukopenia without thrombocytopenia in a man with *equine infectious anemia*.<sup>37</sup>

### Symptoms and Signs

The initial clinical picture in patients with pancytopenia varies widely. The onset is often insidious. Manifestations depend on the severity of the anemia, thrombocytopenia, or leukopenia. Other clinical features and simple laboratory findings reflect the underlying disease process and usually serve to reduce the number of possible diagnoses quickly. Thus the presence of splenomegaly is compatible with many of the conditions listed in Table 56-1 but calls attention in particular to the possibility of leukemia, the lymph node disorders, myelofibrosis, and congestive splenomegaly. The presence of enlarged lymph nodes further supports the possibility of leukemia, one of the lymphomas, or lupus erythematosus. On the other hand, lack of these signs and absence of evidence to suggest vitamin B<sub>12</sub> or folate deficiency should suggest

multiple myeloma or aplastic anemia. The presence of rouleaux on the blood smear or Bence Jones protein in the urine suggests myeloma. Immature erythrocytes and leukocytes in the blood smear (leukoerythroblastic blood picture, Chapter 57) suggest infiltrative disease in the bone marrow (eg, metastatic carcinoma, leukemia or myelofibrosis) except when there is greatly accelerated blood formation and destruction such as occurs in frank hemolytic anemia.

The anemia is usually normochromic and normocytic, but occasionally it is mildly macrocytic. The leukopenia usually is due to a reduction in the absolute number of cells of the myeloid series and thus there is relative lymphocytosis. However, if the reduction is sufficiently great, lymphocytopenia is found as well.

### Diagnosis

Difficulty in diagnosis arises when atypical features are encountered, for example, when a patient thought to have aplastic anemia is found to have a normally cellular or even hypercellular marrow. One explanation for such a contradictory finding is that the biopsy needle has entered an area in which the bone marrow is regenerating after severe damage; this has been shown to occur after benzol intoxication or irradiation (see page 1747). Another not uncommon dilemma arises when several marrow aspirations are found to be acellular in a patient thought to have leukemia. In most situations a larger marrow sample obtained by trephine or surgical biopsy will solve the problem. In a few conditions, such as congestive splenomegaly or "splenic panhematopenia," the diagnosis is arrived at largely by excluding the other possibilities. Finally, in a few patients no clearly defined syndrome can be recognized.<sup>53</sup>

### Treatment

The treatment of pancytopenia is dictated by the nature of the underlying disease.

## Aplastic Anemia

### History and Definition

The concept of aplastic anemia was introduced in 1888 by Ehrlich<sup>14</sup> who described a rapidly fatal case of severe anemia and leukopenia with associated fever, ulcerated gums, and menorrhagia in a young woman; the platelets were not described. At autopsy there was no active marrow and Ehrlich attributed this to primary depression of marrow function.<sup>14</sup> Chauffard, in 1904, introduced the term "aplastic anemia." Subsequently, case reports and reviews were published without clear definition or agreement concerning the criteria for this diagnosis.<sup>15</sup> In time it came to be recognized that cases similar to but with a more chronic course than that described by Ehrlich may be encountered and an association between aplastic anemia and exposure to a variety of chemical and physical agents ultimately began to be recognized. By 1934, aplastic anemia, although still not clearly defined, was described as a distinct clinical entity characterized by pancytopenia and thought to be the result of depressed bone marrow activity.<sup>15</sup> However, in most of the patients reported, the marrow was not aplastic. The authors suggested that "progressive hypocythemia alone should be sufficient reason for making the diagnosis, provided leukemia can be excluded," but the difficulty in differentiating aleukemic leukemia from uncomplicated marrow depression was well illustrated by the cases that were reported.

The term "aplastic anemia" has been used sometimes as essentially synonymous with refractory anemia<sup>7</sup> or even pancytopenia of any cause.<sup>16</sup> Other terms that have been proposed are *progressive hypocythemia*,<sup>15</sup> *aregeneratory anemia*, *aleukia hemorrhagica*, *pamyelophthisis*, *hypoplastic anemia*, and *toxic paralytic anemia*. In several reviews in the early 1950's<sup>1,5</sup> the name "aplastic anemia" was used only to refer to cases in which no evidence of primary disease capable of producing marrow suppression could be found; in many of these patients the marrow was hypoplastic.<sup>1</sup> As the result of a study of 39

cases we suggested, in 1959,<sup>44</sup> that the term "aplastic anemia" be reserved for cases in which pancytopenia exists, in which there is evidence of decreased production of all the elements of the blood formed in the marrow, including severe hypoplasia or aplasia of the marrow, and in which no evidence is found of a primary disease infiltrating, replacing, or suppressing active hematopoietic tissue. Some confusion persists, however, because patients are encountered in whom the clinical and blood pictures are consistent with a diagnosis of aplastic anemia but the bone marrow is not aplastic.<sup>1,53,114</sup> It is recognized that the morphologic structure of the bone marrow, particularly as seen in a single small aspiration or biopsy sample, does not necessarily accurately mirror total bone marrow function and that a clinical and blood picture resembling the acute disorder described by Ehrlich or even the more chronic forms will not always be accompanied by a completely fatty marrow. However, patients in whom occasional nucleated red cells as well as polychromatophilia and stippling, rather than the classical "aregenerative" picture, are present in the blood are better considered as having some other disorder.

### Pathophysiology

The basic defect in aplastic anemia appears to be a failure of blood cell production that involves erythrocytes, platelets, and leukocytes. It is not entirely clear whether some common stem cell population has been decimated or rendered incapable of repopulating the marrow cell mass (a "seed" or *stem cell deficiency*) or whether the "soil" or marrow environment is unsatisfactory for continued cell production (*micro-environment deficiency*).<sup>45</sup> Evidence can be cited to support either of these pathogenetic mechanisms. The success of marrow transplants in some identical twins<sup>79,4</sup> and the increased incidence of nuclear abnormalities in some patients with idiopathic marrow aplasia<sup>39,2</sup> and of chromosome abnormalities in those with Fanconi's anemia (page 1767), as well as the fact that defects have been found in all three cell lines

in patients with *paroxysmal nocturnal hemoglobinuria* and marrow hypoplasia (see page 958), suggest a defect in stem cells. In addition, the marrow content of stem cells capable of forming colonies on in vitro culture is reduced in patients with aplastic anemia.<sup>27</sup> On the other hand, the failure of marrow infusions to repopulate areas of heavily irradiated marrow (>4,000r) in animals,<sup>23</sup> failure of marrow to regenerate in man after more than 2500 to 3500 rads has been delivered,<sup>109,126</sup> and the marrow hypoplasia observed in starvation<sup>303</sup> and in graft-versus-host rejection<sup>310</sup> suggest that the marrow environment is important and in some instances may be unable to support marrow cell growth. Possibly stem cell deficiency occurs in some subjects, while environmental deficiencies predominate in others.

A classification of aplastic anemia is presented in Table 56-1, group 7.

## Acquired Aplastic Anemia

### Etiology

#### *Chemical and Physical Agents Producing Marrow Hypoplasia*

The chemical and physical agents producing marrow hypoplasia can be divided into two main categories, as shown in Table 56-2 and discussed in the following paragraphs.

AGENTS WHICH REGULARLY PRODUCE MARROW APLASIA WHENEVER A SUFFICIENT DOSE HAS BEEN GIVEN. The effects are more or less predictable and the changes observed in human subjects can be reproduced in experimental animals. Of these agents, benzene and ionizing radiation will be discussed here. Most of the remainder, which include a variety of agents used in cancer chemotherapy such as the nitrogen mustards, antimetabolites, and antimitotic agents (Table 56-2), are considered elsewhere (Chapter 55).

**BENZENE AND ITS DERIVATIVES.** Benzene has been known as a cause of fatal aplastic anemia since Santesson's description (1897) of four

cases in workers in a bicycle-tire factory.<sup>72</sup> Benzene ( $C_6H_6$ ) is a hydrocarbon that is obtained as a by-product in the manufacture of coke. It is also to be found in petroleum distillates, the amount depending in part on the composition of the crude petroleum from which the distillate has been derived.<sup>44</sup> Benzene is used as a solvent for rubber, gum, resins, fats, and alkaloids as well as in the manufacture of drugs, dyes, and explosives. It has been employed in many industries, including the manufacture of artificial leather, natural leather, enamels, rubber, waterproof fabrics, lacquers, shellac, paint removers, bronzing, silvering and gilding liquids, and batteries; in electroplating, lithography and photography, dry cleaning, and leather preparation; in the airplane, linoleum, and celluloid industries.<sup>83</sup> Certain petroleum fractions contain significant quantities of benzene and often are used to clean machinery parts or in cleaning grease from the hands. A benzene derivative (cymene) is present in the exhaust gases encountered in the sulfite pulp industry.<sup>73</sup> Benzene is volatile and consequently is readily absorbed by inhalation in badly ventilated rooms; it is poorly absorbed through the skin.<sup>79</sup> The maximum allowable vapor concentration for industrial usage has been 100 ppm,<sup>72</sup> but this is probably too high.<sup>44,79</sup> In spite of its wide recognition as a myelotoxic agent, benzene remains an important cause of hematopoietic damage, even in the home where it has been used in cleaning agents.

The classic picture of leukopenia, thrombocytopenia, and severe anemia represents only the severe and fatal form of poisoning by benzene. Among exposed workers the most common abnormality reported was anemia (48%). Next in frequency were macrocytosis (47%), thrombocytopenia (33%), and leukopenia (15%).<sup>80,81</sup> In other studies of workers making shoes under unhygienic conditions and exposed to benzene vapor, leukopenia was seen in 9.7%, thrombocytopenia in 1.8%, pancytopenia in 2.7%, and both leukopenia and thrombocytopenia in 4.6%.<sup>70</sup> Anemia was common (33%) but responded completely to iron therapy in the patients so treated. It



**Table 56-2. Chemical and Physical Agents Associated with the Development of Pancytopenia and a Hypoplastic Marrow**

- A Agents that regularly produce marrow hypoplasia and aplasia if a sufficient dose is given**
- 1 Benzene its derivatives (trinitrotoluene) and related agents
  - 2 Ionizing radiation (roentgen rays radioactive isotopes, atomic bombs, etc)
  - 3 Sulfur or nitrogen mustard and congeners (busulfan, melphalan cyclophosphamide, etc)
  - 4 Antimetabolites (antifolic compounds purine or pyrimidine analogues such as 6-mercaptopurine, thioguanine, cytosine arabinoside)
  - 5 Antimitotic agents (colchicine periwinkle alkaloids)
  - 6 Certain antibiotics (daunorubicin adriamycin)
  - 7 Other toxic agents (inorganic arsenic, dichlorovinylcysteine, estrogens)
- B Agents occasionally associated with hypoplasia or aplasia**

<i>Class of Compound</i>	<i>20 or More Reported Cases</i>	<i>Single or Very Few Reports</i>
Antimicrobial agents	chloramphenicol <sup>54</sup>	streptomycin, <sup>260</sup> penicillin <sup>54</sup> methicillin <sup>275</sup>
	organic arsenicals <sup>202</sup>	oxytetracycline, chlortetracycline, <sup>291</sup> sulfonamides <sup>44,289</sup>
	quinacrine <sup>211</sup>	sulfisoxazole (Gantresin), sulfamethoxypyridazine (Kynex) <sup>261</sup> amphotericin B <sup>251</sup>
Anticonvulsants <sup>225</sup>	methylphenylethyldantoin (Mesityl), trimethadione (Tridione)	methylphenylhydantoin (Nuvarone), <sup>221</sup> phenacemide (Pharicrone), <sup>226</sup> Dilantin <sup>445</sup> ethosuximide (Zarontin) <sup>221</sup>
Antithyroid drugs		carbethoxythiomethylglyoxaline (Carbimazole), <sup>254</sup> methylmercaptimidazole (Tapazole), <sup>274</sup> potassium perchlorate, <sup>265</sup> propyl thiouracil <sup>277</sup>
Antidiabetic agents		tolbutamide <sup>257</sup> chlorpropamide <sup>292</sup> carbutamide
Antihistamines		tripelenamina (Pyrbenzamine) <sup>264</sup>
Analgasics	phenylbutazone <sup>230,231</sup>	acetyl salicylic acid <sup>293</sup> indomethacin, <sup>254,261</sup> carbamazepine (Tegretol) <sup>250,272</sup>
Sedatives and tranquilizers		meprobamate, <sup>290</sup> chlorpromazine <sup>284</sup> promazine, <sup>44</sup> chlordiazepoxide (Librium), <sup>278</sup> mepazine <sup>44</sup>
Insecticides		chlorophanothana (DDT), <sup>281</sup> parathion, chlordane pentachlorophenol <sup>281</sup>
Miscellaneous	gold compounds <sup>242</sup>	acetazolamide (Diamox) <sup>289</sup> hair dyes <sup>251</sup> dinitrophenol, <sup>270</sup> thiocyanate, <sup>268</sup> bismuth, <sup>258</sup> 285 mercury, <sup>268</sup> colloidal silver <sup>268</sup> carbon tetrachloride <sup>288</sup> solvents <sup>44</sup>

seems probable that the anemia was an incidental finding and due to unrelated iron deficiency. Other manifestations of poisoning that have been observed are lymphocytopenia, increased reticulocyte count, eosinophilia, immature marrow elements in the circulating blood, leukocytosis,<sup>72</sup> and pseudo Pelger-Huet cells.<sup>70</sup> Evidence of increased blood destruction also has been reported. In one survey, serum bilirubin values were found to be elevated in a third of the subjects.<sup>81</sup> Data furnished to support the state-

ment that polycythemia may occur<sup>72</sup> are not convincing.

There are great variations in susceptibility to benzene poisoning.<sup>72</sup> Evidence of poisoning may appear in a few weeks or only after many years of exposure, or it may not be discovered until the onset of infection long after exposure has ceased. Any degree of exposure is potentially dangerous.

Like the blood picture, which may be of the regenerative or the hemolytic type instead of aplastic, the bone marrow may be found

to be hyperplastic rather than acellular and this can be the case even when the blood shows little evidence of regenerative activity. Extramedullary hematopoiesis has been observed<sup>72</sup> and the complete picture of myeloid leukemia has been described in a few patients.<sup>76,77,88</sup> More often, when extramedullary hematopoiesis has been present, marked splenomegaly and a blood picture of myelophthisic anemia have been found (page 1786).

When a benzene-olive oil mixture is injected in rats, the neutrophilic granulopoietic system is first stimulated.<sup>85</sup> The blood neutrophils have been observed to increase in number and there is hyperplasia of myelocytes in the marrow. Frequent subcutaneous injections may produce a leukemoid picture.<sup>72</sup> The myeloid hyperplasia is followed by destruction of these elements. Degenerative changes occur even more rapidly in the lymphatic tissue than in the bone marrow and lymphocytopenia occurs coincidentally. Erythropoietic tissue is more resistant, but hemolytic anemia develops when large doses are given. Eventually, hypoplasia follows degeneration of stem cells.

When marrow regeneration was studied in benzene-intoxicated and in normal rabbits, some of whose bone marrow had been extirpated, it was observed that, in the latter, sheets of primitive reticular cells and bone trabeculae, then fat cells, and finally myeloid tissue appeared as marrow regenerated.<sup>98</sup> In contrast, in the benzene-intoxicated animals, the extirpated marrow regenerated only to the point of formation of primitive reticular cells and fat cells. This would suggest that benzene may inhibit cell division and maturation beyond the level of the primitive reticular cell. Benzene has been shown to inhibit RNA and DNA synthesis by marrow cells.<sup>92</sup> In studies in which mitosis of living erythroblasts was observed, anomalies in chromosome distribution and other changes were found.<sup>96</sup>

Among substances related to benzene, trinitrotoluene is prominent as a cause of aplastic anemia; this agent may also produce hemolytic anemia. It has had wide use in industry.<sup>75,83</sup> Aplastic anemia also has been

reported following inhalation of fumes of various types of hydrocarbon-containing glues ("glue-sniffing")<sup>94</sup> and cutaneous and oral exposure to kerosene.<sup>82</sup> However, there is only circumstantial evidence that the aromatic compounds found in petroleum distillates, other than benzene, have myelotoxic properties.<sup>44,79,85</sup> Aplastic anemia also has been reported following exposure to heated and vaporized benzene hexachloride (lindane),<sup>86</sup> but this appears to be very unusual since little in the way of hematologic effects was noted in several exposed populations.<sup>90</sup>

**IONIZING RADIATION** The acute destructive effect of ionizing radiation on the bone marrow is well known, as is the fact that excessive exposure may result in severe and even fatal aplastic anemia.<sup>108</sup> Lesser grades of exposure lead to less serious or no detectable changes in the blood.<sup>110</sup>

The mode of action of radiation, the susceptibility of various tissues, and the effects of radiation on the blood and bone marrow were discussed in Chapter 55. Here only certain aspects of the effects of accidental or unsuspected exposure to radioactive substances will be considered.

The use of thorium dioxide as a diagnostic aid in the form of Thorotrast was followed many years later in a few instances by the development of aplastic anemia,<sup>107,118</sup> leukemia, and primary malignant disease in the liver,<sup>102,120</sup> the lung, and at the site of injection.<sup>115</sup> The late effects of radium and mesothorium<sup>100</sup> are related to changes in the bones (radiation osteitis, neoplasms in or near bone). Only minor degrees of anemia have been observed and rarely aplastic anemia. Acute radium poisoning, like that in the radium dial workers, is accompanied by very striking changes in the blood (see below). A high incidence of leukemia and cancer has been observed in persons exposed to ionizing radiations and aplastic anemia has been reported in a few survivors.<sup>112,114</sup>

The effects of continuous internal irradiation were described many years ago in radium dial workers by Martland.<sup>116</sup> In these individuals, injured by the ingestion of radium

through the habit of wetting their brushes by mouth, severe macrocytic anemia with megakaryoblasts in the circulating blood, leukopenia, and relative lymphocytosis developed. The femur bone marrow was dark red and showed primitive red cells and leukocytic hyperplasia, as well as numerous megakaryocytes. In a number of subjects there was necrosis of the jaw and buccal lesions similar to those found in patients with acute leukemia. Low-grade osteitis, replacement fibrosis of the marrow, and osteogenic sarcoma were also encountered. In some of the affected workers, death occurred as late as four to six years after leaving their occupation. In experimental radium poisoning<sup>122</sup> the first effect of radioactive substances given orally was the production of changes in the teeth, mouth, and jaws, changes similar to those found in dial workers.<sup>104</sup> Hyperplastic marrow, similar to that described by Martland, and macrocytic anemia have also been noted in human beings and animals subjected only to external roentgen radiation.<sup>108,110</sup>

The practice of making regular blood counts in persons occupationally exposed to radiation has little to justify it.<sup>119</sup> Degrees of radiation exposure twenty times greater than the maximal permissible dose may produce a real drop in neutrophils or lymphocytes, but the change is slight and serious aplastic anemia has been observed to occur without necessarily being preceded by much decrease in leukocytes.<sup>119</sup> Regular physical measurement of the doses received by the use of film badges and other methods is a much more practical procedure in most situations.

**OTHER TOXIC AGENTS** *Inorganic arsenic* in the form of potassium arsenite once was employed in the treatment of leukemia because of its consistent depressing effect on the leukocyte count. In experimental studies, decreased red cell production resulted from the administration of arsenic and arsenious acid.<sup>120</sup> In inorganic arsenic poisoning, granulocytopenia,<sup>121</sup> anemia, and, less often, thrombocytopenia<sup>120</sup> occur and basophilic stippling is a prominent finding.<sup>128</sup>

The feeding of *extracted soybean meal* has

been shown to produce aplastic anemia in cattle and several other species,<sup>141</sup> apparently as a result of a toxic product (*dichlorovinylcysteine*) formed by the interaction of trichloroethylene used to extract the oil and cysteine in the beans.<sup>142</sup> Also very large doses of *estrogens* have produced hypoplastic anemia in dogs.<sup>135</sup>

**AGENTS OCCASIONALLY ASSOCIATED WITH MARROW HYPOPLASIA OR APLASIA. HISTORY.** There is an ever-increasing number of compounds (Table 56-2) that are only occasionally associated with bone marrow hypoplasia or aplasia. Not only is their number great, but their deleterious effect is unpredictable. The basis for assuming that they are the cause of untoward reactions is circumstantial, since it is only when a particular drug has been associated with some adverse reaction repeatedly that an etiologic relationship can be strongly suspected. For example, suspicion that the occurrence of aplastic anemia and the taking of chloramphenicol might be more than coincidental<sup>184</sup> led to the chain of events that culminated in the establishment of the Adverse Reactions Registry of the American Medical Association. From 1949 until 1952, when its use was unrestrained, chloramphenicol was the drug most frequently associated with the development of aplastic anemia in the United States.<sup>41</sup> A sharp decrease in the total number of cases appearing annually followed public warning of the hazard in 1952, but this was of brief duration because of denials of a possible relationship. The number of cases reported to the Registry rose progressively to a peak of 83 cases in the year 1959. Since then in the U.S.A. there has been a progressive decrease.<sup>56</sup>

Experience with chloramphenicol illustrates well the nature of the problem involved in adverse reactions to drugs. Where one deals with a reaction the incidence of which is very low, it is natural that there should be skepticism concerning the etiologic relationship between the drug and the reaction. Furthermore, it is difficult when examining case reports to check the validity of the his-

tory of drug intake or the hematologic diagnosis, and patients treated with one drug are likely to have received one or more other drugs. Thus to separate the potentially noxious from the innocent drugs is difficult. However, in an analysis of drugs reported to the AMA Registry as being associated with the development of pancytopenia, chloramphenicol was the drug most commonly associated with this reaction and in a striking proportion of instances it was the only drug given, or it was given with drugs not known to be toxic (Fig. 56-1). The contrast between the reports involving chloramphenicol and, for example, sulfonamides is illustrated in Figure 56-1. In the greater proportion of instances in which sulfonamides were named, these drugs had been given together with a potentially toxic one. This would imply that the sulfonamides were not etiologically related to the reaction observed.

The proportion of reports for a specific dyscrasia in relation to a particular drug also has been helpful in the analysis of data available to the Registry. Of 2149 cases reported

to June 30, 1964, 32.2% were concerned with pancytopenia, 17.2% with thrombocytopenia. Of the blood dyscrasias attributed to chloramphenicol, however, 70.3% were cases of pancytopenia, and only 6.5% were thrombocytopenias. Such a disproportionate concentration of reports of a specific dyscrasia in relation to a drug is further reason to suspect an etiologic relationship. In the same analysis, as many as 87.3% of the reports concerning quinidine referred to thrombocytopenia and only 3.2% reported pancytopenia. This strongly suggests a relationship of quinidine to thrombocytopenia; in the case of quinidine, there is evidence of another type incriminating it as an etiologic agent in thrombocytopenia (page 1092)

**MECHANISMS OF TOXICITY** From a study of case reports of aplastic anemia associated with drug administration, it seems clear that the observed ill effect is a result of individual sensitivity or susceptibility since no clear association with the amount of drug taken or with frequent or excessive usage has been

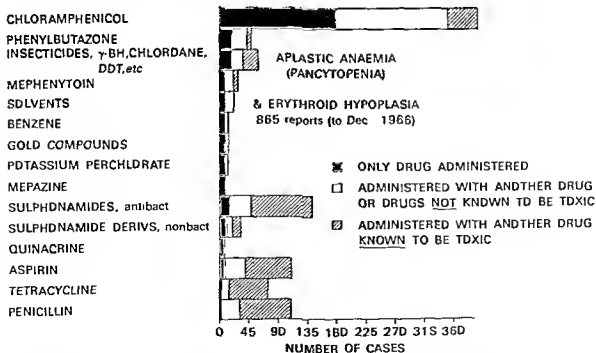


Fig 56-1. Numbers of cases of aplastic anemia and erythroid hypoplasia reported to the Registry on Adverse Reactions, Council on Drugs AMA (From Wintrobe,<sup>36</sup> courtesy of the author and Journal of the Royal College of Physicians)

demonstrated. As yet, no reliable method for predicting drug sensitivity has been devised, and no inherited enzymatic defects such as those resulting in hemolytic anemia on exposure to certain drugs (page 779) have been discovered to explain the individual sensitivity. Attempts to produce similar changes in animals have met with failure.

**INCIDENCE** The incidence of aplastic anemia associated with drug administration is difficult to determine. In respect to quina-crine (Atabrine), when a large number of soldiers were given this drug for malaria prophylaxis, aplastic anemia developed in about 1 in 25,000 man-years or in 1 to 50,000 persons<sup>211</sup> as compared to 1 in 500,000 soldiers not so treated.<sup>187</sup> In regard to chloramphenicol, the risk of developing fatal aplastic anemia may be in the range of 1 in 60,000<sup>182</sup> or 1 in 20,000<sup>187</sup> i.e., about 13 times the frequency of aplastic anemia in the population not so exposed.<sup>187</sup> For most other drugs, the incidence is unknown since the population at risk has not been determined. Of 1067 cases of aplastic anemia reported to the American Medical Association Council on Drugs, 376 of the patients had received chloramphenicol and this was the only drug given to 173 of the group.<sup>56</sup> Compared to chloramphenicol, other drugs appear to be relatively infrequent offenders (Fig. 56-1). A few drugs have been reported as being associated with aplastic anemia in 20 or more persons, while a large number have only rarely been reported as being associated with this dyscrasia (Table 56-2). Many of these drugs may also be associated with the occurrence of neutropenia alone (page 1292), thrombocytopenia (page 1084), or "bicytopenias."<sup>2</sup> In a number of instances these disorders have been reported more often than has aplastic anemia. For example, phenothiazines, sulfonamides, antihistamines, and anti-thyroid drugs are more commonly associated with neutropenia than with aplastic anemia, whereas chloramphenicol<sup>151</sup> and the organic arsenicals have been more frequently associated with aplastic anemia.

*The agents only occasionally associated with*

aplastic anemia can be considered in groups according to drug class (Table 56-2). Only the more frequent offenders will be discussed in any detail.

**CHLORAMPHENICOL** An analysis of 408 cases of chloramphenicol-associated, non-oculoplastic depression of one or more blood cell types reported to the Registry showed persons of all ages to be affected, there being a broad adult range of from 25 to 65 years old and a childhood peak.<sup>182</sup> Until the menopausal age, 70% of the subjects were females. These trends are at least in part explained by patterns of occurrence of diseases for which chloramphenicol is often given. The significance of a high incidence among Caucasians and, in particular, north Europeans, is unclear, but may be related to the care with which patients have been studied and to the degree of willingness to report blood dyscrasias. In 75% of the reported patients, all three blood cell types were depressed and marrow hypoplasia was found. Chloramphenicol had been given in most instances for treatment of, or prophylaxis against, infection, and in many instances the indication for its use was of dubious validity. No clear relationship could be established between the development of the dyscrasia and previous exposure to the drug, the dose employed, or continuous, as compared with intermittent, administration. In 50% of the subjects, evidence of reaction appeared within 38 days of the last dose, in 22% during therapy, and in 10% after 130 days. In an occasional patient, aplastic anemia developed after administration of as little as 2 g of drug to a patient never before so treated.<sup>185</sup> The overall mortality rate was about 50%, half of the deaths occurring within 50 days of the reaction. Variables speaking for a more favorable prognosis were (1) fewer blood cell types depressed; (2) non-Caucasian race; (3) development of reaction during therapy or shortly thereafter; and (4) cases treated with large daily doses.

As already noted, the pathogenesis of chloramphenicol-associated blood dyscrasias is obscure. Although not all investigators agree, most believe that two types of reac-

tions occur, a reversible suppression of erythropoiesis and irreversible aplasia.<sup>152,190,193</sup> If so, it is very possible that they are unrelated.

Reversible bone marrow suppression primarily involving erythropoiesis occurs in about 50% of patients to whom chloramphenicol is given in large doses. Increased serum iron, reticulocytopenia, and a falling level of hemoglobin may be found.<sup>162,175,177</sup> Ferrokinetic studies made at this time reveal prolonged half-disappearance time of plasma iron, decreased uptake of radioiron by the bone marrow, increased uptake by the liver, and failure of radioiron to appear in circulating red cells for eight days or more.<sup>175</sup> Thus, suppression of heme and hemoglobin formation<sup>189</sup> has been demonstrated. Less often, neutropenia and thrombocytopenia may develop.<sup>170,175</sup> The marrow is not characterized by hypoplasia, and abnormal sideroblasts have been seen in the marrow in some patients at this stage.<sup>150</sup>

A striking finding in the bone marrow is vacuolization of the nucleus and cytoplasm of the erythroblasts<sup>177</sup>; similar changes occur in the granulocytic series (Fig. 56-2) although less frequently.<sup>162</sup> These toxic effects usually have been observed between 11 and 18 days after the antibiotic was started and have been correlated with elevated levels of serum chloramphenicol concentration<sup>180</sup> and impaired clearance of the drug.<sup>185</sup> If the drug is withdrawn at this point the reaction is reversible.<sup>180</sup> It is reported that susceptibility to this form of chloramphenicol toxicity can be predicted if clearance of a single 500 mg dose of drug is found to be delayed.<sup>185</sup>

It was claimed that the administration of l-phenylalanine to children caused the cellular vacuolization to disappear and remain absent in spite of continued administration of chloramphenicol,<sup>185</sup> but a similar effect was not noted in adults.<sup>160</sup>

This reversible and primarily erythropoietic toxicity from chloramphenicol ap-



Fig. 56-2. Vacuolated erythroblast and myelocyte in the marrow of a patient with aplastic anemia apparently due to chloramphenicol. (Courtesy of Dr. Ralph Wellerstein)

pears to be a pharmacologic action of the drug. Its biochemical basis has been the subject of some controversy. Initially it was proposed that chloramphenicol is bound to messenger RNA and thus ribosomal protein synthesis is blocked.<sup>159,161</sup> However, subsequent studies could not confirm this,<sup>160</sup> but provided evidence that mitochondrial protein synthesis and mitochondrial ultrastructure of both erythroid and myeloid cell precursors are adversely affected by the usual clinical levels (10 to 16  $\mu\text{g/ml}$ ) of chloramphenicol.<sup>161</sup>

Chloramphenicol also inhibits the post-phagocytic respiratory burst in human leukocytes, perhaps by inhibiting NADH oxidase activity<sup>162</sup>; in rabbits a chloramphenicol-induced defect in erythroid precursor respiration was reported to be due to a depletion of mitochondrial cytochromes.<sup>158</sup> There also is evidence that the drug retards the bio-transformation of drugs such as tolbutamide, diphenylhydantoin, and dicoumarol by inhibiting the microsomal drug-metabolizing system in the liver.<sup>153,155</sup> The latter observations may explain the impression that patients chronically ill with liver disease are especially likely to develop chloramphenicol toxicity.<sup>162</sup>

The relationship of such erythropoietic depression by chloramphenicol to aplastic anemia is unknown. Some believe that erythropoietic depression is a basically different and reversible toxicity.<sup>191,194</sup> However, the occasional production of pancytopenia by high-dose therapy<sup>170</sup> or by the more toxic chloramphenicol analogues (Fig. 56-3),<sup>190</sup> the production of vacuoles in myeloid as well as erythroid forms, and the evidence that more immature cell forms such as primitive erythroblasts<sup>191</sup> seem more sensitive to chloramphenicol toxicity than mature cell forms suggest that similar toxicity at a stem cell level may be the cause of aplastic anemia. The question remains unanswered.

*Severe and often irreversible bone marrow aplasia*, frequently resulting in death, is the second type of reaction to chloramphenicol. As already mentioned, there is equivocal evidence that this reaction is related clinically or pathogenetically to the reversible, mainly

erythropoietic lesion described above. Individual susceptibility seems a more likely basic mechanism. The concordant development of aplastic anemia in one set of identical twins<sup>173</sup> and the development of aplastic anemia in one of another set of twins with thrombocytopenia in the partner<sup>156</sup> suggest the presence of an heritable predisposition that may have to be present in homozygous form before susceptibility is manifest.<sup>152</sup> Since, in most instances, aplastic anemia becomes evident only after use of the drug has been discontinued and since a long period of aplasia follows even in those patients who recover, some type of damage that lingers long after the last traces of drug have disappeared must be considered. Damage to a high percentage of stem cells has been postulated.<sup>152</sup> Damage to the chromosomes of human blood leukocytes cultured *in vitro* in the presence of chloramphenicol in usual therapeutic concentrations (10 to 40  $\mu\text{g/ml}$ ) provides support for this concept.<sup>171</sup>

There is no evidence that monitoring the effects of chloramphenicol by means of repeated blood counts will reduce the incidence of chloramphenicol-associated aplastic anemia. Far more important is the initial judgment and restraint of the physician who prescribes the drug. With this, as with other potentially harmful therapeutic agents, the physician must weigh the risk against the possible gain. All too often chloramphenicol has been prescribed for trivial reasons.

**ORGANIC ARSENICALS.** Organic arsenicals (arsphenamine, neoarsphenamine, mapharsen, etc.), once the agents of choice in the treatment of syphilis, are known to cause a variety of blood dyscrasias, more commonly thrombocytopenia or neutropenia but also aplastic anemia.<sup>200,201</sup> The development of aplastic anemia was unrelated to the dose administered or the duration of therapy, and the proportion of patients who developed such anemia was small. However, because of the once widespread use of arsenical therapy, more than 50 cases of aplastic anemia associated with such treatment were reported.<sup>204</sup> Sulfarsphenamine was probably the most

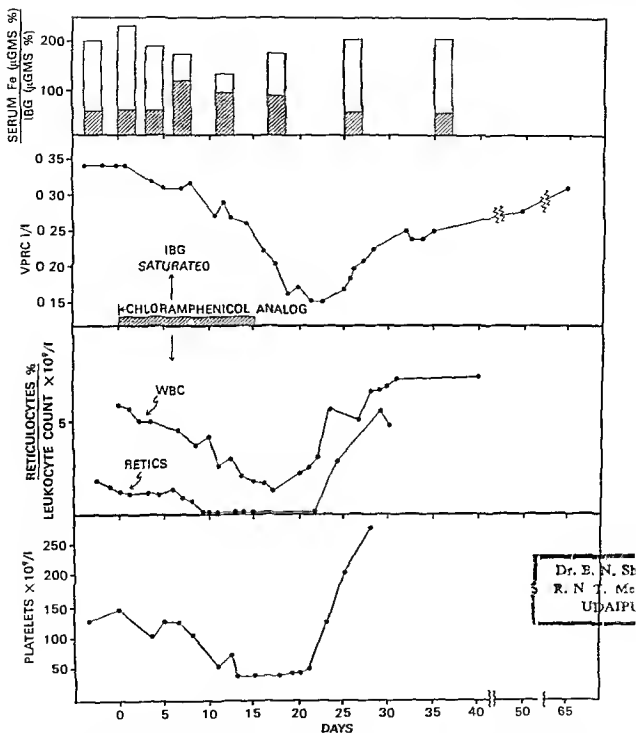


Fig 56-3. The effect of a chloramphenicol analog on serum iron and peripheral blood counts. Similar effects are seen with chloramphenicol itself. IBG denotes iron binding globulin. (From Weisberger,<sup>100</sup> courtesy of the author and the Journal of the American Medical Association.)

dangerous and Mapharsen the least toxic of the agents employed. Cross-reactivity between agents was not always demonstrable and it is not clear whether the toxic effect

was due to the benzene ring, the arsenic, the combination, or to sensitization. Danger signals in patients receiving these drugs were thought to be itching, skin rash, ready bruising.



ing, or fever or malaise appearing soon after injection.<sup>205</sup> The use of BAL did not prove beneficial in the few patients so treated.<sup>206</sup>

**QUINACRINE (ATABRINE).** Fifty-seven fatal cases of aplastic anemia secondary to the use of this drug for malaria suppression were seen in the South Pacific during World War II. As mentioned above, the incidence was estimated at 1 in 20,000 as compared to much lower levels (1 in 500,000) in troops in other theaters not so treated.<sup>211</sup> Aplastic anemia has also been reported in patients with discoid lupus treated with quinacrine.<sup>210,217</sup>

The mechanism of quinacrine toxicity is unknown. However, in rabbits the drug is concentrated in the marrow and lymphoid tissues from which its disappearance is considerably delayed as compared to its disappearance from the blood; also human marrow cells concentrate the drug *in vitro*.<sup>215</sup> Quinacrine suppresses leukocyte respiration *in vitro*<sup>214</sup> and leukocytes from patients treated with a related drug (chloroquine) were found to contain myelin figures (perhaps autophagic vacuoles) when examined with the electron microscope.<sup>213</sup>

**ANTICONSULSANTS** More than 48 cases of aplastic anemia associated with anticonvulsant drug therapy, especially methylphenylhydantoin (Mesantoin) and trimethadione, have been reported.<sup>220</sup> The onset of the anemia occurred as early as 2 weeks or as long as 30 months after starting treatment, but in most instances after 4 to 13 months. There was no relation to age, sex, or drug dosage.<sup>225</sup>

The mechanism of the toxic effect of these drugs is unclear. In one patient with pure red cell aplasia due to diphenylhydantoin the dyscrasia disappeared after drug withdrawal and recurred on two subsequent occasions when therapy was reinstituted for 17 and 32 days, respectively.<sup>445</sup> Additional studies in this patient suggested that the drug interfered with deoxyribotide formation in erythroid precursors, thereby inhibiting DNA synthesis.<sup>470</sup>

**PHENYLBUTAZONE.** The number of reported cases of aplastic anemia associated with use of this drug is difficult to determine but is at least 35 to 40.<sup>5,23,233</sup> Neutropenia is a much more frequent toxic effect than is aplastic anemia, but it has been claimed that the incidence of severe toxic reactions of all kinds is low in patients treated with doses of less than 200 mg/day.<sup>235</sup> The mechanism of action is unclear but has not been carefully studied.

**GOLD.** When gold compounds were in popular use for the treatment of rheumatoid arthritis and to a lesser extent for lupus erythematosus and tuberculosis, toxic effects were frequent, occurring in as many as 42% of one large series of 900 patients.<sup>241</sup> However, hematologic toxicity was noted in less than 5% of patients, and thrombocytopenia and granulocytopenia were more common than aplastic anemia. Nevertheless, the fatality rate in aplastic anemia due to gold was high, only 4 of 20 patients surviving,<sup>241</sup> and several patients were treated with BAL without perceptible benefit.<sup>240</sup> An increase in the use of gold in the treatment of rheumatoid arthritis seems likely as a result of the demonstration of its effectiveness in double blind trials, and the appearance of new cases of associated blood dyscrasias can be expected.<sup>242</sup>

**OTHER DRUGS AND AGENTS.** In addition to the above-named drugs, a wide variety of agents has been reported only very rarely as being associated with aplastic anemia. Many of these are listed in Table 56-2.

### *Other Causes of Aplastic or Hypoplastic Anemia*

Pancytopenia with aplastic or hypoplastic marrow has been reported occasionally in association with such conditions as *mycobacterial infection*<sup>15</sup> and, with increasing frequency, in infectious *hepatitis*.<sup>30,32,41,43</sup> Several varieties of dengue type *virus infections* in Southeast Asia have been reported to be associated with hypoplastic anemia,<sup>300</sup> and

about 50 cases were reported in association with pregnancy, most of the subjects improving after delivery or abortion.<sup>305,313</sup> Rare cases have been reported in association with sclerosis of the thyroid gland,<sup>308</sup> *Simmonds' disease*,<sup>302</sup> anorexia nervosa,<sup>311</sup> and graft-versus-host (runt) disease.<sup>307</sup> Marrow hypoplasia secondary to the ingestion of *contaminated grain* has been reported in Russia and was thought to be due to a toxin (perhaps a coumarin compound) produced by a mold.<sup>44</sup>

### "Idiopathic" Aplastic Anemia

Although one may search carefully for a possible etiologic agent in cases of pancytopenia, there remain approximately 50% of cases in which no cause is found or suspected.<sup>44,370</sup>

### Symptoms and Signs

In the majority of patients the initial symptoms of acquired aplastic anemia are manifestations of anemia and/or bleeding, but fever or infection are not uncommon.<sup>54</sup> The onset is insidious. The symptoms and their character depend on the rapidity with

which the anemia progresses and whether or not complicating infections and hemorrhage develop. Infection and hemorrhage, in turn, depend in part on the degree of granulocytopenia and thrombocytopenia. If the course is rapid, fever and symptoms attributable to anoxemia arise. If it is slow, progressive weakness and fatigability are the chief complaints until the thrombocytopenia becomes well marked, when bleeding from the nose, mouth (Fig. 56-4), or gastrointestinal tract, menorrhagia, or purpura develops. In general, however, purpura is not the most conspicuous feature. Ulcerations in the mouth and pharynx or low-grade cellulitis in the neck appear late, as a rule. In idiopathic cases the illness is often dated from an attack of some common type of febrile disorder. In the secondary forms, symptoms may develop some weeks or even several months following exposure to the causative agent. The first sign of benzene poisoning may appear with the onset of an infection, long after exposure has ceased.<sup>72</sup>

A waxy pallor is usually well marked by the time attention has been drawn to the illness. The yellowish tint of pernicious anemia is lacking. Weight loss is unusual. There may be bruises or purpuric spots in the skin,

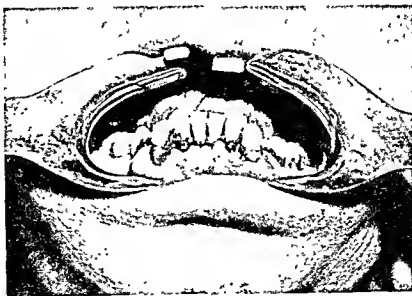


Fig 56-4. Bleeding gums in a patient with aplastic anemia

but these are often inconspicuous in relation to the degree of thrombocytopenia. Hemorrhages may be noted in the eyegrounds. Splenomegaly is so unusual that, when the spleen is palpable, "aleukemic" leukemia or some other condition that may simulate aplastic anemia should be looked for. It should be noted, however, that, in a few patients with benzene poisoning,<sup>72</sup> the spleen was found to be enlarged. The tongue shows no papillary atrophy. Changes in the nervous system are found only when hemorrhage has occurred there, although there may be some complaint of paresthesias in this as in other forms of anemia. The skin may show a brownish pigmentation.<sup>7</sup>

### *Blood Changes*

As a rule, the red corpuscles are more or less normal in appearance in spite of the severity of the anemia. Polychromatophilia, stippling, and nucleated red corpuscles are usually not found and the uncorrected reticulocyte count is normal or low.<sup>54</sup> In some instances, however, the anemia has been macrocytic, there has been moderate anisocytosis and poikilocytosis,<sup>325</sup> and immature red cell forms have been reported.<sup>45</sup> The finding of young erythroid forms suggests an error in diagnosis for, even in patients in whom the bone marrow seemed to be hyperplastic, the circulating blood often gave no sign of regeneration.

The hemoglobin may be as low as 7 g/dl and the volume of packed red cells 0.2 l/l or lower when the patient is first seen.<sup>54</sup> At the same time there usually is leukopenia and thrombocytopenia. The leukocytes formed in the bone marrow are affected chiefly and thus the smear may contain as many as 70 to 90% lymphocytes. The leukocyte count may, however, be as low as  $1.5 \times 10^9/l$  or even  $0.15 \times 10^9/l$  and thus absolute lymphocytopenia is present as well. In patients in whom immature erythrocytic forms have appeared in the circulating blood, occasional immature myeloid leukocytes have been observed, but one would hesitate to make the diagnosis of aplastic anemia in such patients.

The bleeding time is usually moderately prolonged and the blood clot retracts poorly when thrombocytopenia is present. Coagulation parameters generally are normal. The fragility of the red corpuscles is normal.

The serum iron usually is elevated and the iron-binding protein saturated. Free erythrocyte protoporphyrin has been found to be increased.

Ferrokinetic studies in a patient with typical aplastic anemia are illustrated elsewhere (Fig. 4-10, page 166). The plasma radioiron clearance rate ( $t_{1/2}$ ) is prolonged, there is decreased uptake of radioiron by the marrow and an increase in liver and spleen uptake, whereas the incorporation of iron into circulating erythrocytes is decreased. Red cell life span may be somewhat shortened,<sup>325,327</sup> but evidence in the plasma, urine, and stools of increased blood destruction is lacking. In some patients, evidence of splenic sequestration of red cells has been reported.<sup>325</sup> In some of the other pancytopenic states, more variable results for plasma iron clearance and different ferrokinetic parameters have been reported<sup>4724</sup> and these may help in distinguishing such cases from aplastic anemia.

Hemoglobin F levels may be elevated in children with aplastic anemia and a separate population of hemoglobin F-containing erythrocytes can be identified in blood smears stained by the acid elution method.<sup>322</sup> Presumably such cells are produced by a clone of cells formed in an attempt to compensate for the severe anemia. In one series of children with acquired aplastic anemia, most of those with hemoglobin F levels greater than 400 mg/dl of red cells survived, whereas the majority with levels less than this died.<sup>322</sup> However, later studies did not confirm this observation.<sup>371</sup>

That granulopoiesis may be qualitatively as well as quantitatively abnormal is suggested by the finding of abnormal granulation in the neutrophils and high leukocyte alkaline phosphatase.<sup>375</sup>

Erythropoietin levels are markedly increased in patients with hypoplastic or aplastic anemia.<sup>329</sup>

## Bone Marrow

In patients with the classical type of acquired aplastic anemia the material obtained on sternal puncture consists chiefly of mature red corpuscles. The majority, even 60 to 100%, of the nucleated cells are lymphocytes. Such a finding makes it advisable to obtain a larger specimen, by biopsy, in order to be sure that one has obtained true bone marrow and not blood and also to see how fatty the marrow is. However, the most important bone marrow finding is the proportion of cells that are nonmyeloid and are not erythroblasts, since the proportion of such cells was found to be directly related to mortality.<sup>51,371,351</sup>

In marrow biopsy specimens from patients with typical aplastic anemia, only yellowish-white material, consisting chiefly of fat, fibrous tissue, and lymphocytes, is seen (Fig. 56-5). Marrow that appears red macroscopically may be found to be composed largely of extravasated blood and shows little evidence of regeneration. However, as pointed out previously, this classic picture is not always found in cases that otherwise seem to fit the category of aplastic anemia. In some patients there may be a preponderance of lymphocytes. In still others,<sup>35,53</sup> but especially in those with benzene poisoning<sup>72</sup> and in some who have had internal<sup>116</sup> or external<sup>114</sup> irradiation, the marrow has been hyperplastic. It is difficult to give the relative numbers of patients with aplastic, hypoplastic, normal, or hyperplastic bone marrow. This depends largely on the definitions and criteria used in diagnosis and has varied greatly in different series of subjects.<sup>355</sup>

## Diagnosis

From the hematologic standpoint, the characteristic triad is anemia, leukopenia, and thrombocytopenia. However, as outlined in Table 56-1, the causes of pancytopenia are numerous and those other than aplastic anemia must be ruled out before the diagnosis of aplastic anemia is made. The finding of lymphadenopathy or splenomegaly makes a

diagnosis of aplastic anemia very unlikely although, as mentioned earlier, slight splenic enlargement has been described in some patients (even 10% of one series<sup>54</sup>), especially after repeated transfusions. In addition to a thorough physical examination, which must include rectal examination and careful palpation of all the bones for tenderness, extensive radiographic studies may be required to rule out the possibility of lesions in bones or tumors not evident on physical examination. Examination of the urine cannot be overlooked as it may give evidence of multiple myeloma or of renal carcinoma. If fever is present, conditions such as Hodgkin's disease must be considered even though fever of slight or even moderate degree may occasionally accompany aplastic anemia, especially the more fulminating forms. A remote possibility is military tuberculosis (page 1742).

The finding of immature forms of the red or white series in the circulating blood is strong evidence against aplastic anemia, although here again exceptions have been reported. In patients in whom the anemia is macrocytic rather than normocytic and in whom some signs of red cell regeneration are present, real difficulty may be encountered. The possibility of pernicious anemia can be ruled out by the absence of glossitis, achlorhydria, or neural involvement, by the lack of pigmentary evidence of increased blood destruction, by the absence of true megaloblasts in the bone marrow, and, if the diagnosis is still in doubt, by the serum B<sub>12</sub> level or the Schilling test, as well as by the failure of appropriate therapy. Other forms of megaloblastic anemia are discussed on page 574. The possibility of hemolytic anemias should be excluded; for example, paroxysmal nocturnal hemoglobinuria may be mistaken for aplastic anemia. In aplastic anemia, the leukopenia is characteristically due to neutropenia. If all types of leukocytes are reduced in number or if lymphocytes are reduced to a greater degree than the neutrophilic series, Hodgkin's disease and even disseminated lupus erythematosus must be considered.

Thorough inquiry must be made concern-



Fig 56-5 Sections of bone marrow from patients with aplastic anemia compared with those of normal subjects

A Normal sternal marrow. Note the presence of disseminated erythropoietic foci (small hyperchromatic cells) and several scattered megakaryocytes (large cells with abundant cytoplasm).

B, Marked hypoplasia. Small foci of sparse cellularity composed of lymphoid or erythropoietic elements are observed.

C Moderate hypoplasia. An overall reduction in cellularity is apparent, the degree of cellular pleomorphism seen in this photograph represents the presence in reduced numbers of both myeloid and erythroid elements in various stages of maturation. A focus of small lymphocytes is present.

D Higher magnification of the lymphoid follicle present in C. Small lymphocytes predominate. The larger cells with more abundant cytoplasm are primitive reticular elements. (From Scott, Cartwright, and Wintröbe "courtesy of the authors and Williams & Wilkins Co.)

ing possible exposure to potentially toxic agents, either at the patient's place of work or at his home, in his pursuit of hobbies, or through medication whether prescribed by his physician or self-administered. The number of agents that may damage the hematopoietic system is large (Table 56-2) and growing.

The presence of only minimal neutropenia and thrombocytopenia should cause one to consider seriously the presence of some underlying chronic disease such as one of the conditions discussed in the first part of this chapter. A form of anemia that may be mistaken for aplastic anemia is that associated with chronic renal disease because this ane-

mia is notoriously unresponsive to therapy and the manifestations of the renal disease may be overlooked. A diagnosis of "pure red cell" aplasia (page 1769) must be made with caution for such cases are rare.

The diagnosis of aplastic anemia is one that is made largely by exclusion. Even when bone marrow puncture yields little marrow, the diagnosis is not certain for, from some patients with leukemia or with metastatic disease of the bone marrow, it may be difficult to withdraw any diagnostic material. Marrow puncture, even when repeated at several sites, may be misleading; we have demonstrated active marrow by surgical biopsy even when puncture repeatedly yielded very few cells. Needle biopsy of the bone marrow (page 66) is easily performed and often extremely helpful (Fig. 56-5).

Bizarre normoblastic chromatin or megaloblastoid cells are noted in patients with the di Guglielmo syndrome (page 1474) and these may alert one to this diagnosis even before the preponderance of early leukocytic forms characteristic of acute leukemia becomes evident. Similar chromatin abnormalities are present in patients with Fanconi's anemia<sup>132</sup> (page 1767). The finding of abnormal sideroblasts suggests sideroachrestic anemia (page 678), while extensive fibrous tissue, tumor nodules, or granulomas may suggest myelofibrosis, myelophthisic anemia, or other disorders (Chapter 57).

The differentiation of thrombocytopenic purpura (page 1071) should cause no difficulty. Although there may be considerable similarity on clinical grounds, the anemia that may be found in thrombocytopenic purpura is only proportional to the degree of blood loss. The leukocyte count should be normal or increased and signs of red cell regeneration may be found if there has been much loss of blood. Likewise "agranulocytosis" (page 1290) may have to be considered on clinical grounds, but in this condition anemia is slight or absent and thrombocytopenia is not found. Occasionally, as illustrated in Figure 56-6, only anemia or leukopenia, thrombocytopenia, or "bicytopenia" may be the first pre-

senting sign, only to be followed ultimately by pancytopenia with a hypocellular or acellular marrow.

The estimation of red cell production by means of ferrokinetic studies (page 164) provides data that are useful as an indication of decreased erythropoiesis, but this requires considerable time and effort and cannot be regarded as an essential diagnostic tool. Sometimes, however, it is useful to measure red cell survival with <sup>51</sup>Cr and to determine whether there is excessive destruction in the spleen.

### Treatment<sup>396</sup>

Treatment involves (1) a thorough search for a possible cause and prohibition of further exposure to a suspected drug or other toxic agent even if the evidence is not very impressive; (2) maintenance of hemoglobin levels by blood transfusion; (3) prevention and management of hemorrhage and infection; (4) attempts to stimulate hematopoiesis and marrow regeneration; (5) evaluation of the possible role of the spleen in the destruction of transfused red cells or platelets, and the removal of this organ if the evidence suggests that this may be wise and the patient can tolerate the procedure; (6) possible marrow transplantation in carefully selected patients.

### Avoidance of Further Exposure

Although it is obvious that the first requirement is the avoidance of further exposure to an etiologic noxious agent, this is sometimes overlooked or the cause is not recognized. It is essential that a thorough and systematic search be made. Ideally the noxious agent, if still present, should be removed from the body, but this is not always possible. When the toxic agent is an arsenical, gold, or certain radioactive isotopes, BAL (British anti-Lewisite), penicillamine, or other chelating agents may be tried. These agents will enhance excretion of metals<sup>365</sup> but clinical benefit is variable<sup>206,210,241</sup> and perhaps unrelated to the treatment.<sup>244</sup>

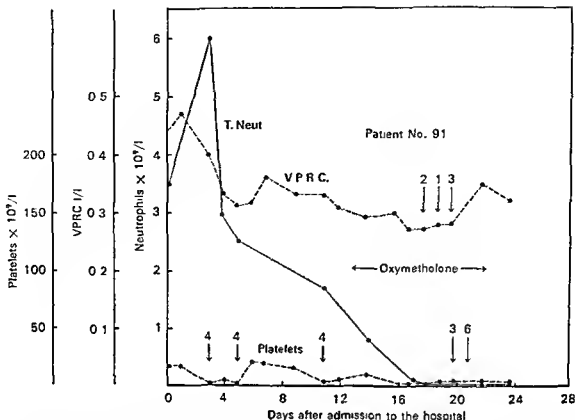


Fig 56.6. Course of a patient with acute aplastic anemia whose first manifestation was thrombocytopenia 20 days following oxyphebutazone ingestion. Biopsy specimen obtained three days after hospital admission revealed hypocellular bone marrow. The patient subsequently became anemic, neutropenic, and died. The numbers indicate the units of red cells or platelets transfused. (From Williams et al.,<sup>54</sup> courtesy of the authors and Seminars in Hematology.)

### Relief of Anemia

The mainstay of treatment has been the transfusion of whole blood for the relief of anemia. Currently specific blood components (leukocyte-poor packed red cells, platelet-rich plasma or concentrates, and less often leukocyte concentrates) are being used in many centers both to more effectively utilize the available blood supplies and to minimize side effects such as isoimmunization and undesirable blood volume expansion<sup>357</sup> (Chapters 11, 12). Whether whole blood or component therapy is given, transfusions should be used in moderation and with considerable care for several reasons. First, it must be recognized that most patients without associated problems such as ischemic heart disease can tolerate a packed red cell volume of 0.3 l/l, or

even lower, quite satisfactorily. Thus it is unnecessary as well as costly and impractical to attempt to keep a patient's hemoglobin at normal levels by blood transfusion. Second, the bone marrow failure may not be complete, in which case it may be found that the hemoglobin will stabilize at a satisfactory level or may decrease only slowly so that transfusion is needed infrequently. Third, the transfusion requirement is quite constant in most patients and the first indication of bone marrow recovery may be a decrease in the rate of hemoglobin decline, permitting a prolongation of the interval between transfusions. In this regard it is useful to transfuse with the same amount of blood at regular intervals and to record the subsequent rate of hemoglobin fall. With such a routine, evaluation of myelostimulative treatment is

facilitated (see below). Fourth, since each unit of blood or of packed or washed red cells contains 200 to 250 mg of iron, problems associated with iron overload will arise in time.<sup>339</sup> The use of desferrioxamine in an attempt to alleviate this problem has proved disappointing.<sup>379</sup> Finally, the risks of transfusion, including hepatitis, febrile reactions, and other complications, increase with the number of units given. For all of these reasons the number of transfusions should be kept to a minimum.

### ***Prevention and Management of Hemorrhage and Infection***

**Bleeding and/or purpura** due to thrombocytopenia are common initial findings, occur at some time during the course of the illness in practically all subjects, and are a common cause of death.<sup>44,54</sup> Hemorrhage due to causes other than thrombocytopenia, though unusual, does occur and therefore the possibility of other causes should be ruled out. Serious hemorrhage is infrequent with platelet counts above  $20 \times 10^9/l$  as judged from experience with patients with acute leukemia.<sup>356</sup> Based on these findings it is common practice, especially when hemorrhage is a problem, to administer fresh blood,<sup>367</sup> platelet-rich plasma, or platelet concentrates in amounts sufficient to maintain the platelet concentration above  $20 \times 10^9/l$ .<sup>349,354,400</sup> However, some patients do not bleed even at levels lower than this. If the blood is collected in plastic bags with ACD as the anticoagulant, any of these forms of treatment is effective; best results are obtained with platelet-rich plasma or platelet concentrates prepared so that the pH is maintained between 6.5 and 6.8.<sup>333,354</sup> Minimal centrifugation and rapid processing are especially important. An average increment of  $12$  to  $14 \times 10^9$  platelets/l/meter of recipient body surface area has been reported when  $1 \times 10^{11}$  isologous platelets (about the content of one unit of platelet-rich plasma) were transfused.<sup>354</sup> The presence of infection or active bleeding decreases this increment considerably. Although isoimmunization to platelets

occurs and markedly decreases the effectiveness of therapy,<sup>358</sup> this is seldom a problem until at least 10 transfusions have been given. The incidence of isoimmunization increases steadily from about 5% with less than 10 transfusions to 37% after 50 to 100 and to 80% after more than 100 transfusions.<sup>390</sup> In patients who require repeated platelet infusions the isoimmunization problem may be circumvented by using a limited number of donors<sup>390</sup> or, even better, HL-A compatible parents or siblings.<sup>400</sup> It has been estimated that a median of 4.5 units/m<sup>2</sup>/week of HL-A compatible platelets will prevent bleeding in patients with aplastic anemia and such therapy has been used for as long as two years.<sup>358,400</sup> Other prophylactic measures such as suppression of menstruation by the administration of oral contraceptives, the avoidance of aspirin or other drugs that inhibit platelet function, the use of mouth washes or water piks rather than coarse toothbrushes and the like, may avoid the initiation of bleeding.

**Infection** resulting from leukopenia and neutropenia is a common cause of morbidity and mortality in aplastic anemia<sup>54,370,397</sup>; it also aggravates the thrombocytopenia and appears to precipitate bleeding in many instances.<sup>397</sup> In general, the more serious infections seem to occur in patients with low neutrophil counts.<sup>397</sup> Although neutrophil transfusions cannot be effectively used to support patients on a long-term basis because of the short survival of neutrophils in the blood,<sup>335</sup> such transfusions may be useful in helping a patient survive an acute infectious process.<sup>368</sup> However, in such a special situation, neutrophil replacement therapy is difficult because insufficient cell numbers can be obtained from normal donors (ideally HL-A compatible family members), even if cell separators are available. A possible alternative is to use isologous cells from patients with chronic myelocytic leukemia.<sup>338,350</sup> In spite of considerable investigative effort, the place of leukocyte transfusions in the management of aplastic anemia remains uncertain.<sup>396</sup>

Exposure to infections should be avoided



if possible. This may involve the restriction of visitors, reverse isolation, or the use of plastic isolators or laminar air-flow units.<sup>368</sup> However, many infections are derived from the patient's own bacterial and fungal flora. In an attempt to eliminate this source, in addition to meticulous mouth care, non-absorbable broad-spectrum antibiotics and special low-bacterial or sterile diets have been given to sterilize the bowel. The effectiveness of these regimens is under investigation; they appear to be promising.<sup>368,383</sup> The use of absorbable prophylactic antibiotics (with the exception of isoniazide [INH] in appropriate patients) is not recommended since this encourages the development of resistant strains and atypical infections. When fever or infection does develop, a prompt and vigorous search for the site and cause should be instituted. This should include careful physical examination (with rectal examination); chest film and other x-ray studies when indicated; and culture (anaerobic, aerobic, fungal, etc) of blood, sputum, urine, stool, suspicious skin or subcutaneous lesions, including possible anal fistulas, and, in many instances, of spinal fluid and bone marrow. After such a vigorous search, but sometimes based only on the results of smears stained for bacteria, if the situation seems critical, vigorous therapy with appropriate bactericidal agents should be instituted; this can be changed later as dictated by culture and sensitivity results.

### *Stimulation of Hematopoiesis and Marrow Regeneration*

Of the measures employed to improve marrow function the most widely used has been administration of anabolic steroids (testosterone, oxymetholone, etc).<sup>330,347,376,386,387,389,392</sup> There is no agreement regarding the agent of choice. Some advocate the use of non-17 $\alpha$ -alkylated agents such as testosterone or methyl testosterone<sup>347</sup> because they are cheaper than many of the other agents and there are few side effects such as impairment of liver function.<sup>387</sup> On the other hand, a few patients refractory to testosterone have responded to oxymetholone.<sup>330</sup> The dosage

used has varied considerably, but, at least initially, methyl testosterone, testosterone propionate, or oxymetholone, 1 to 2 mg/kg/day, is given orally. There is some evidence to suggest that responses may occur somewhat sooner with these than with lower doses.<sup>387</sup> Since no certain way of selecting those patients who will respond has been discovered,<sup>387</sup> all patients with aplastic anemia should be given a trial of such therapy. If fluid retention or other side effects develop they will often abate with reduction of drug dosage; side effects disappear for the most part when use of the drug has been discontinued. The injection of parenteral preparations such as testosterone enanthate should be avoided if possible, but these have been reported to be effective and can be used in certain patients if necessary.<sup>342</sup>

A rise in hemoglobin, ultimately even to 12 g/dl or more, can be expected in about 50% of patients in some series,<sup>387,389</sup> but the response is slow (Fig. 56-7). In other series, androgens seemed less beneficial.<sup>371</sup> Reticulocytosis may develop after four to six weeks of treatment, followed usually by stabilization of the hemoglobin level and then a rise toward normal; in a few patients the response may begin earlier (two to four weeks) or later (five to six months). A rise in neutrophil concentration usually occurs in those patients whose hemoglobin rises, but the response is slower and less marked than is the rise in hemoglobin. Neutrophils have returned to normal in only about 35% of patients. The platelet response is even less than the neutrophil response (about 25% return to normal).<sup>387</sup> Permanent remission has been reported in about two thirds of patients who have survived long enough to receive an adequate trial on androgen therapy. In those patients with acquired aplastic anemia in whom remission occurred, the remission usually persisted after therapy was discontinued (in contrast to patients with constitutional aplastic anemia, see page 1768).

Adrenal corticosteroids have been used extensively in the treatment of patients with aplastic anemia and occasional good reticulocyte responses and improvement have been

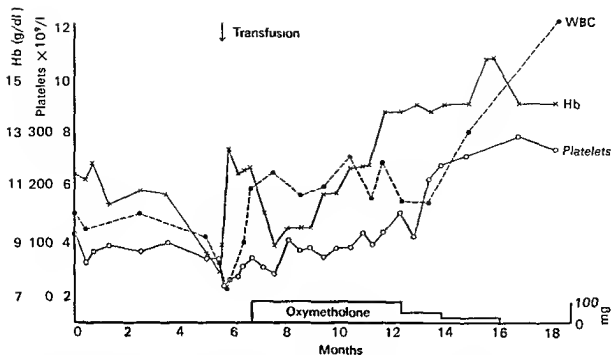


Fig 56-7. Aplastic anemia, showing response to oxymetholone (From McCredie,<sup>376</sup> courtesy of the author and the British Journal of Haematology)

reported (Fig. 56-8). However, the remission rate with corticosteroid therapy alone is only about 12% and the use of corticosteroids in combination with androgens does not appear to improve results. Thus some investigators believe that corticosteroids add little to therapy.<sup>387</sup> In children, however, they are recommended for their alleged effect in counteracting androgen-induced bone maturation<sup>347,388</sup> and they may be useful in patients with uncontrollable bleeding.<sup>396</sup> Cobalt therapy has not proved useful in the treatment of patients with aplastic anemia.<sup>347</sup> Phytohemagglutinin has been used in an attempt to stimulate mitosis and marrow regeneration with apparent good response in some subjects.<sup>331,360,364</sup> The usefulness of this agent in therapy has not been adequately evaluated but, after initial enthusiasm, interest appears to have subsided.<sup>360</sup>

### Splenectomy

Splenectomy has been advocated on two grounds: (1) withdrawal of an inhibitory effect on hematopoiesis and (2) removal of

a site where red cells and platelets may be sequestered or destroyed.<sup>352</sup> Our experience and that of others<sup>44,361,373,386</sup> has been that the operation may be helpful in some patients (Fig. 56-9). However, critical evaluation of reported results, including our own, did not provide conclusive evidence of benefit from the operation.<sup>44,54</sup> The patients who seemed to benefit might have recovered spontaneously. If shortened red cell or platelet survival can be demonstrated and if the spleen appears to be related to this as judged from isotope studies (page 1419), the operation deserves serious consideration. In general we have employed splenectomy when the condition has been chronic, other measures have failed, no evidence of spontaneous recovery has been apparent, and when it appeared that the operation might reduce the need for transfusions or reduce their number. It has been suggested that splenectomy is more likely to be helpful if the bone marrow is not completely aplastic and if corticosteroids have been associated with some evidence of response.<sup>373</sup> A return to normal blood levels is unusual, although this occurred in 2 of 39...

ment.<sup>370,371</sup> Death ensues in a few weeks or life may linger as long as six months. On the other hand, about half the patients survive for well over a year, some for four, five, or more years. Of 39 patients originally reviewed by us, 50% lived for two and one half years and 40% survived for four years or longer (Fig. 56-10). Our more recent study of 99 patients has further emphasized the biphasic shape of the survival curve.<sup>374</sup> Patients who survived for four months or less differed from long survivors in mode of onset, interval from onset of symptoms to first clinic visit, and initial hematologic values. Hemorrhagic symptoms and rapid clinical deterioration, with early seeking of medical attention, were characteristic of the short survivors. More than 70% non-myeloid cells in the initial bone marrow study correlated with high mortality in this and in other series.<sup>34,371</sup> Patients with very low initial reticulocyte counts ( $< 0.5\%$ ) or profound neutropenia (less than  $0.25 \text{ cells} \times 10^9/\text{l}$ ) have done poorly.<sup>34,370,371,387</sup> The alleged prognostic value of fetal hemo-

globin level, however, has been disputed.<sup>371</sup>

Fatal forms of aplastic anemia have been more frequent in young than in older subjects according to some<sup>347,391,389</sup> but not other<sup>363</sup> investigators. In one study the outlook was found to be more serious in patients over the age of 40.<sup>370</sup> These contradictory statistics, in addition to the differences in the course of patients with aplastic anemia, cited above, illustrate the difficulty of evaluating prognosis and new therapeutic regimens in a disease with a variable course. They may explain the differences in opinion regarding the value of androgen therapy in aplastic anemia.<sup>54,344,370,371,384</sup> If reliable criteria for prediction of prognosis can be developed,<sup>374</sup> the selection of patients for whom bone marrow transplantation (page 1764) should be recommended may be facilitated.

Acute leukemia has developed as a terminal event in patients with aplastic anemia<sup>336,341,370</sup> and paroxysmal nocturnal hemoglobinuria has also been reported as a complication.<sup>370,385</sup> In an unusual case, fea-

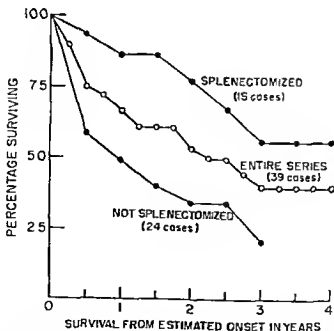


Fig 56-10. Percentage survival in 39 patients from estimated onset of aplastic anemia (From Scott, Cartwright, and Wintrobe<sup>44</sup> courtesy of the authors and Williams & Wilkins Co)

tures of PNH developed in a patient with aplastic anemia refractory to treatment, with final termination in acute myeloblastic leukemia.<sup>398</sup> The relationship of these diseases to one another is as yet unclear.

### Constitutional Aplastic Anemia

Constitutional aplastic anemia is associated with congenital defects, or hematologic abnormalities present since infancy, and familial occurrence. This type of aplastic anemia is believed to be due to inherited, rather than acquired factors. Several apparently different clinical entities are recognized.

#### *Fanconi Syndrome (Congenital Pancytopenia)*

Under the title of "familial, infantile pernicious-like anemia," Fanconi described a fatal disorder in three brothers that was characterized by pancytopenia, bone marrow hypoplasia, and congenital anomalies. More than 165 cases have now been reported.<sup>413,418,424</sup> The anemia is normocytic or slightly macrocytic, and macrocytes and target cells may be present in the blood. Reticulocytes may be slightly increased in number (6 to 10%), but the absolute reticulocyte count is reduced; occasional immature forms of red or white cells have been noted in blood smears. No evidence of a hemolytic process was noted in the earliest reports (except one case<sup>415</sup> that seems more probably to have been one of paroxysmal nocturnal hemoglobinuria), but since then shortened red cell survival has been described in some subjects.<sup>418,421</sup> In most of the patients the leukopenia has been due to neutropenia, but, in several, all the varieties of leukocytes were affected equally. The bone marrow has been described as fatty, hypocellular, normally cellular, and even hypercellular, and may contain many plasma cells and mastocytes.<sup>418</sup> An elevated fetal hemoglobin level appears to be a consistent feature.

A patchy, brown pigmentation of the skin, due to the deposition of melanin, is a common finding in Fanconi's anemia. Other features of the disorder include dwarfism; mi-

crocephaly; hypogenitalism; strabismus; anomalies of the thumbs, radial bones (Fig. 56-11), and kidneys; mental retardation; and microphthalmia.<sup>426</sup> Atrophy of the spleen is common. Congenital vascular anomalies are unusual.<sup>410</sup> Somewhat more common in males than in females (2:1) the anemia has generally been detected in the first eight years of life, rarely as late as the third decade. There appears to be no racial or geographic preponderance, but in a number of instances several siblings have been affected. Siblings of patients with the complete syndrome have had congenital anomalies without hematologic manifestations. The cause of the disorder is unknown but the syndrome is generally thought to be hereditary, perhaps due to a recessive gene, or the result of reciprocal chromosomal translocation in one of the parents and a duplication deficiency in the affected offspring.<sup>418</sup> Cytogenetic studies have revealed a variety of structural aberrations and a specific type of polyploidy,<sup>411</sup> but the significance of this is still uncertain.<sup>428</sup> Cultured lymphocytes from patients with Fanconi's anemia show a high prevalence of chromosomal breaks (Fig. 56-11)<sup>423,428</sup> and skin fibroblasts exhibit increased susceptibility to malignant transformation by SV-40 virus.<sup>423</sup> Consanguinity of the parents has been noted a number of times<sup>420</sup> and chromosomal abnormalities have been reported in both parents of one affected family.<sup>416</sup> A possible *forme fruste* in the mother with hypoplastic anemia in her offspring was reported.<sup>427</sup> Attention has been called to the similarity between the thalidomide embryopathy and Fanconi's anemia.<sup>434</sup> The high incidence of leukemia that has been noted in the families of patients with Fanconi's anemia<sup>420</sup> may possibly be related to the chromosomal aberrations that have been described.<sup>418</sup> Some cases of congenital hypoplastic thrombocytopenia (page 1100) may progress to Fanconi's pancytopenia, and there appears to be a close relationship to dyskeratosis congenita.<sup>413,432</sup>

The treatment of patients with Fanconi's anemia is similar to that of those with acquired aplastic anemia (page 1759). Most pa-

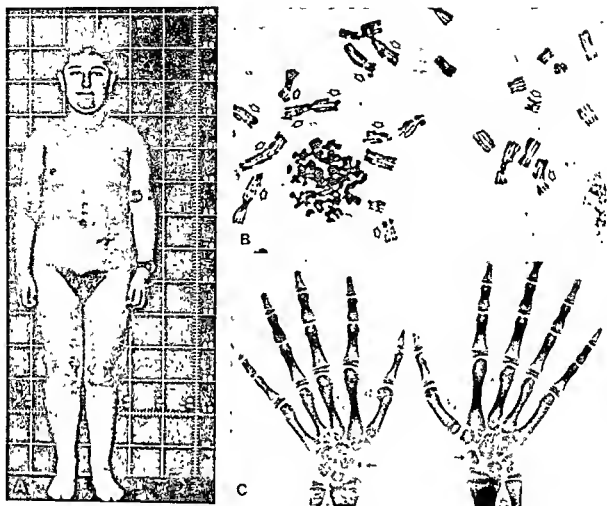


Fig 56-11. Features of Fanconi's anemia. A An 11½ year old boy with Fanconi's anemia in whom the onset of the disease was at age six. Splenectomy was performed because of chronic panmyelocytopenia. Treatment with prednisone, testosterone, and Danabol permitted this patient to have a nearly normal life, but with Cushingoid changes and masculinisation. B, A portion of a squash preparation from a lymphocyte culture of a patient with Fanconi's anemia to illustrate endoreduplication of chromosomes and chromatid breaks (indicated by the arrows). C X rays of the hands of the boy in A. The left thumb was slightly smaller than the right, and an abnormal metacarpal bone indicated by the arrow is seen in the left hand (From Fanconi<sup>418</sup> and Schmid,<sup>428</sup> courtesy of the authors and Henry M. Stratton, Inc.)

tients with Fanconi's anemia will respond to androgen and corticosteroid therapy.<sup>347</sup> Since the bone marrow in patients with this disorder usually contains some cells, the response may be more rapid with an earlier and higher reticulocyte peak than in patients with acquired aplastic anemia. However, the final response is really no better, in terms of degree of hemoglobin increase, than in acquired aplastic anemia. The neutrophil concentration increases in most patients, but the

platelet response is much less consistent. In contrast to acquired aplastic anemia, patients with Fanconi's aplastic anemia need continuous, maintenance androgen therapy.<sup>347</sup>

#### *Aplastic Anemia with Pancreatic Insufficiency*

A few patients with pancytopenia, bone marrow hypoplasia, and associated, grossly deficient pancreatic enzyme secretion were

discovered in a cystic fibrosis clinic.<sup>430</sup> These patients differed from the patients with cystic fibrosis in that their sweat tests gave normal results and they had few if any respiratory tract difficulties. Three patients were from one family. No treatment has proved effective.

## "Pure Red Cell" Aplasia<sup>464</sup>

Erythropoietic hypoplasia occurring in the absence of abnormalities in the leukopoietic or thrombocytopoietic systems is often referred to as "pure red cell" aplasia. This condition may appear as an acquired defect of either acute or chronic type, but a congenital familial form is noted as well.

### Acquired Acute Erythropoietic Hypoplasia

In hereditary spherocytosis (page 752) and in other hemolytic anemias (page 723), and sometimes in children in the course of various infections<sup>453</sup> or in association with malnutrition,<sup>458</sup> the erythroblasts may suddenly disappear from the bone marrow for a short time (*acute erythroblastopenia*).<sup>442</sup> If the aplastic crisis persists, anemia will develop. Acute arrest of erythropoiesis also has been reported in adults following relatively minor infections<sup>442,446</sup> and 39 cases of pure red cell aplasia developing in association with various types of *drug therapy* have been reported.<sup>470</sup> The drugs incriminated include aminosalicylic acid, glutethimide, aspirin, butabarbital, colchicine, heparin,<sup>452</sup> sulfathiazole, arsphenamine, diphenylhydantoin,<sup>445</sup> isoniazide, chenopodium, tolbutamide, and chlorpropamide.<sup>454,470</sup> In most of these patients, complete recovery followed withdrawal of the drug.

### Chronic Acquired Erythropoietic Hypoplasia

Chronic acquired erythropoietic hypoplasia appears to be of several types. It is not uncommon in certain parts of the world, particularly in children, and has been attributed

to malnutrition, chronic infection, and vitamin deficiency.<sup>450,458</sup>

A clinically similar *acquired erythrocytic hypoplasia* or "pure red cell" aplasia occurs in adults. About 150 cases have been reported<sup>463</sup> and it has been suggested that several forms may exist, namely, those associated with benign thymoma<sup>452,456,475</sup> and those unassociated with demonstrable thymic abnormality<sup>443,448,466,467,472,476,477</sup>; a few cases also have been noted in association with miscellaneous malignant diseases.<sup>449,469</sup>

In more than 50% of patients, predominantly women (3 to 4.5:1), a thymoma has been demonstrable.<sup>448,473</sup> Myasthenia gravis was noted in 14% of the patients in one series<sup>473</sup>; thymectomy, performed in 15 patients, provided immediate hematologic improvement in four but no benefit in the remainder.<sup>473</sup> In occasional patients thymectomized for myasthenia gravis, pure red cell aplasia has developed as long as three years postoperatively.<sup>448</sup> In patients with *pure red cell aplasia without associated thymoma*, males predominate over females 2 to 1.

The age of the patients has ranged from 20 to 67 years, but the majority have been in the fifth to seventh decades of life. In spite of the above-described apparent subgroups, no very clear classification of pure red cell aplasia is possible. The anemia is chronic, usually severe, and normocytic or slightly macrocytic. Reticulocytes are decreased or absent and leukocyte and platelet counts are usually normal although, in a few subjects, leukopenia or thrombocytopenia has developed.<sup>463,464</sup> The marrow is usually cellular with normal granulocytopoiesis and thrombocytopoiesis but with a paucity of erythroblasts. The cause is obscure but the prevailing hypothesis is that an antibody inhibits erythropoiesis. Supporting this concept is the demonstration of an IgG antibody to erythroblast nuclei in several patients with uncomplicated pure red cell aplasia.<sup>462,463,464,465</sup> An inhibitor of *in vitro* heme synthesis was found in patients without or with associated thymoma.<sup>440,457</sup> A few patients in both groups have responded to treatment with adrenal steroids, androgens, or immunosup-

pressive therapy.<sup>443,448,462,463,464,469</sup> In a few patients the anemia appears to have begun in childhood; a relationship to the congenital disorder of infancy may be suspected.<sup>460</sup>

### Constitutional Erythroid Hypoplasia

Probably several varieties of constitutional erythroid hypoplasia exist, but as yet only one or two forms are recognized.

In 1938, *Diamond and Blackfan* described a *syndrome* that they characterized as a slowly developing and progressive anemia, beginning early in infancy, in which white blood cells and platelets were normal. In the bone marrow only red cells were deficient.<sup>490</sup> Since the initial description other, apparently identical, cases have been described under a variety of names, such as erythrogenesis imperfecta,<sup>488</sup> chronic congenital aregenerative anemia (pure red cell anemia),<sup>502</sup> and chronic erythroblastopenia.<sup>490</sup> Pallor may be first noted at birth and is usually apparent before the infant is one year of age.<sup>490</sup> Hepatomegaly and/or splenomegaly were present in about 40% of the patients in one series, but both the liver and the spleen were observed to shrink after transfusion, thereby suggesting that their initial increase in size reflected heart failure. Minor congenital anomalies have been observed in a few (7 of 30 patients), but no renal abnormalities were present, thus perhaps further differentiating this disorder from Fanconi's anemia. No icterus was present, nor was there other evidence of increased blood destruction.

The anemia is severe (Hgb 1.7 to 9.4 g/dl) and normochromic, normocytic in type.<sup>493</sup> The reticulocyte count usually is less than 1% and normoblasts are not found in the blood smear. As already mentioned, leukocyte and platelet counts usually give percentages within the normal range (except later in a few cases, when splenomegaly may develop after many transfusions). Hemorrhagic manifestations do not occur. The bone marrow on biopsy appears normal except for a gross deficiency of erythroid precursors.

The course is insidious and progressive,

but 20% of patients in one series developed spontaneous remissions, sometimes after as long as eight months to 13 years of failure to respond to any form of treatment.<sup>490</sup> Corticosteroid therapy induced remission in 12 of 22 patients in whom this therapy was tried; in three of the 12, remission persisted without medication.<sup>490</sup> Similar benefit has been reported by other investigators.<sup>498,500</sup> In those patients not responding to treatment, blood transfusions have been necessary to sustain life, but the transfusions have been responsible for the most serious complication of this disease, namely, transfusion siderosis. Either the continuing hemosiderin deposition or subclinical hepatitis produces severe liver damage. Growth retardation, failure of sexual maturation, osteoporosis, and portal hypertension, perhaps resulting from the effects of anemia and hemosiderosis, are common in these patients. Splenectomy has been helpful in only a very occasional subject and should probably be reserved for patients who are unresponsive to corticosteroid therapy and those who develop leukopenia, thrombocytopenia, and shortened erythrocyte survival after many transfusions.

The cause of the failure of erythropoiesis is unknown. A familial incidence has been observed in some subjects and this, together with the very early age of onset, suggests that the abnormality is genetically determined.<sup>490</sup> In one patient, hypocalcemia and a chromosomal abnormality were described.<sup>503</sup> Reports of this condition in several patients born of different mothers but having the same father suggest dominant transmission of the defect (and expression in heterozygotes); for unknown reasons, males appeared to be less affected than females and thus perhaps sometimes survive without the disease having been detected.<sup>497</sup> In three patients, adenine nucleotides in the plasma and red cells were increased.<sup>504</sup> In some patients an abnormality in tryptophan metabolism was described,<sup>493,490,498</sup> but it is not clear that this was related to the disease.<sup>499</sup>

*Triphalangeal thumbs and red cell aplasia* were reported in several male members of one family.<sup>489</sup> These patients were similar to

patients with Fanconi's anemia in that they had radial hypoplasia (although mild), but the onset at birth (rather than at seven or eight years), the absence of skin pigmentation, and the limitation of hematologic changes to the erythroid series clearly distinguishes them from those with Fanconi's anemia. Triphalangia of the thumbs was a consistent feature.

From time to time *other forms* of constitutional erythroid hypoplasia that have not been consistent in all respects with the clinical pictures outlined above have been described. In one series, congenital anomalies were such as to suggest Fanconi's anemia, but only the erythroid series was affected. The marrow showed variation in cellularity, the number of erythroblasts being decreased, normal, or increased; defects of maturation also were noted in several.<sup>495</sup> In another patient, intermittent jaundice and anemia together with hepatosplenomegaly, low reticulocyte count, erythroid hyperplasia in the marrow, and multinucleated normoblasts appeared to be related to excessive ("double") cytoplasmic membranes in the normoblasts and ineffective erythropoiesis.<sup>507</sup> In several other persons the congenital anomalies were like those in the Fanconi disorder but the bone marrow was hyperplastic.<sup>492</sup>

Aplastic anemia and lymphocytosis, together with hypogammaglobulinemia, depletion of lymphoid tissue, and histiocytosis of spleen, lymph nodes, and bowel, were described in a three and one-half month old infant. This was thought to resemble the "runting syndrome" caused by a graft-versus-host reaction.<sup>493</sup>

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## Myelofibrosis

### Idiopathic Myelofibrosis

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### History

Heuck<sup>40</sup> probably was the first to recognize that IMF differs from leukemia, emphasizing that the two patients whom he reported in 1879 were distinguished from those with CML by the intensity of the fibrosis in the bone marrow and the well-organized nature of the extramedullary hematopoietic tissue of the spleen and liver. The first careful description of a small series of cases was published by Meyer and Heineke in 1907.<sup>68</sup>

### Etiology and Pathogenesis

As implied by its name, the cause of IMF is unknown. Many authors have considered this to be a neoplastic disease,<sup>12,25,62,68,71</sup> but there is little evidence for morphologic or chromosomal cellular abnormality in most patients<sup>9,89</sup> and the similarity of secondary forms of MF to IMF suggests that it need not be neoplastic. However, the presence of a defect in patients with IMF<sup>58</sup> that is somewhat similar to that seen in erythrocytes in patients with paroxysmal nocturnal hemoglobinuria (PNH) has been described. The membrane abnormality in patients with PNH suggests a clonal, hematopoietic stem cell defect (Chapters 2 and 29). Of 22 patients with IMF whose cells were examined, positive reaction was obtained to the Ham test in one, to the sucrose hemolysis test in 12, and to the sugar water test in 10.<sup>58</sup> Other

**F**IBROSIS of the bone marrow (myelofibrosis) may occur as an idiopathic syndrome in which extramedullary hematopoiesis usually is present as well, or it may develop in association with a variety of diseases, principally infections and tumors (page 1786).

### Idiopathic Myelofibrosis (IMF)

In its typical form, IMF can be defined as a fairly discrete disorder. At least 37 different terms have been applied to this disease<sup>14</sup>; the most frequently used synonym probably is *agnogenic myeloid metaplasia*.<sup>49</sup>

investigators<sup>42,51,59,89</sup> have considered IMF to be due to an unidentified, abnormal stimulus that results in excessive proliferation of fibroblasts and osteoblasts as well as blood cells. It has been demonstrated that an excessive number of granulocytic stem cells can be found in the blood of patients with IMF,<sup>20</sup> but whether this indicates that there is an excess of total number of stem cells in the body is uncertain. Thus it is unknown whether the bone marrow fibrosis is a secondary reaction to undefined injury or a primary element of an unidentified proliferative abnormality involving fibroblasts as well as hematopoietic cells. It is noteworthy that in one patient in whom a consistent chromosome defect was demonstrable in myeloblasts, this could not be seen in the fibroblasts.<sup>86</sup> It has been suggested that the increase in fibrous tissue may simply represent a response to increased hematopoiesis since a similar change can be observed in the hematopoietic foci in the spleen and liver.<sup>37,41</sup> However, if this were the case, marrow fibrosis should be anticipated in patients with chronic hemolytic anemia, but it is rarely, if ever, found in these patients. Saponin, injected intravenously, regularly leads to production of myelofibrosis in the rabbit and in this model damage to the microvasculature of the bone marrow appears to be the initiating event.<sup>68a</sup>

### Incidence

The incidence of IMF is not known, but estimates based on the relative frequencies of IMF and CML<sup>13,56,89</sup> would place it at between 0.2 and 2 patients/100,000 population. IMF is considered to be much more common in Caucasians than in Negro,<sup>89</sup> Japanese,<sup>76</sup> or Mexican American<sup>89</sup> populations. There are no convincing reports of familial IMF.<sup>89</sup> The incidence in males and females is approximately equal, a summation of 14 published series yielded a total of 292 males and 275 females.<sup>89</sup> IMF is primarily a disease of the middle-aged and elderly, the average age at diagnosis being approximately 60, but cases in infants<sup>77</sup> have been reported.

### Features of Disease at the Time of Diagnosis<sup>12,42,55,59,68,71,80,89</sup>

There is evidence to suggest that IMF often has been present for many years before the diagnosis is made. One of our patients was known to have a palpably enlarged spleen 15 years prior to diagnosis, and Ward and Block<sup>89</sup> reported that this also was the case in 3 of 45 patients. On the basis of the average observed rate of increase in spleen size in untreated patients, Ward and Block estimated that the disease had been present for one year for each centimeter that the spleen extended below the costal margin<sup>89</sup> (Fig. 57-1). However, the rate of change in the size of the spleen is so variable from one patient to another (Fig. 57-1) that such calculations, while interesting, are very crude approximations at best.

### Symptoms

The diagnosis often is made in asymptomatic patients following investigation of abnormal blood findings or because of the discovery of splenomegaly. The patient may note a mass (spleen) in the abdomen, but

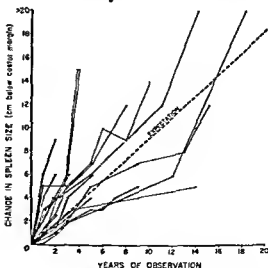


Fig 57-1. Changing spleen size with time during the natural course of IMF as observed in 16 patients (From Ward and Block,<sup>89</sup> courtesy of the authors and Withams & Wilkins Company.)

other symptoms attributable to splenomegaly are infrequent unless splenic infarction has occurred. Weight loss may be noted; in some patients it may be the major complaint, usually associated with fatigue. In turn, the presence and degree of fatigue often reflect the degree of anemia. Bleeding, most often manifested only by easy bruisability, occurs in a few patients. Gout may be present and renal colic also may be an initial complaint, reflecting hyperuricemia. Other symptoms noted by a few patients include fever and diarrhea, the cause of which is usually obscure.

### Physical Findings

The only finding in the majority of patients is splenomegaly.<sup>12,42,53,59,68,71,81,89</sup> It is not uncommon for the spleen to be so large that its lower border is below the pelvic brim and its right border protrudes across the midline of the abdomen. Even when barely palpable, the spleen usually is felt to be of very firm consistency. Absence of a palpable spleen is quite rare, but does not rule out the diagnosis of IMF. Hepatomegaly is found in approximately 50% of the patients. Petechiae, ecchymoses, and lymphadenopathy are detected in a few. In contrast to its frequency in patients

with CML, sternal tenderness is unusual in patients with IMF, at least in most series, although, in one series,<sup>55</sup> it was said to be present in almost half of the patients. Deafness due to otosclerosis has been suggested as being associated with IMF more frequently than would be expected by chance.<sup>74a,89</sup> Pallor may be detected, depending on the presence and degree of anemia, and jaundice may be observed in a few of the patients. Acute arthritis (gout) may be present, but gouty tophi are unusual.

### Laboratory Findings

**THE BLOOD.** *Anemia* is present in the majority of patients at the time of diagnosis but varies greatly in severity. In general, it can be said that anemia becomes more severe as the disease progresses. Polycythemia may be observed in occasional patients in whom no evidence for preexisting polycythemia vera is present.<sup>89</sup> Red cell indices generally are normochromic, normocytic, but examination of the blood smear reveals abnormal red cells in most, but not all, patients (Fig. 57-2). The classic findings are teardrop-shaped and nucleated erythrocytes; a careful search reveals at least a few of the latter in the blood smears

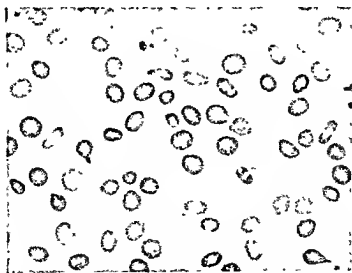


Fig. 57-2. "Teardrop" polukocytes from a patient with myelofibrosis (Wright's stain  $\times 720$ )



of almost all of these patients.<sup>80</sup> Nucleated erythrocytes may be so frequent in the blood that the "leukocyte" count is misleading unless these cells are subtracted from the count, since the latter includes all the nucleated cells. Fragmented, target, and polychromatophilic cells may be observed and increased reticulocytes may be present. Rarely, megaloblastic anemia is encountered.<sup>31,33</sup>

Kinetic studies of the anemia reveal it to be of complex causation. The plasma volume may be increased and occasionally the total red cell mass may be found to be normal<sup>46</sup> in "anemic" patients with IMF.<sup>17,21,63,89</sup> Red cell survival is decreased in almost all patients and increased levels of indirect bilirubin may be present. Evidence for autoimmune hemolysis is found only rarely.<sup>68,89</sup> Of the intrinsic erythrocytic enzyme defects known to be associated with a shortened red cell survival in patients with other diseases (Chapter 20), none has been detected in patients with IMF, but red cell G6PD, 6-phosphogluconic dehydrogenase, and reduced glutathione may be increased.<sup>6,31,33</sup> As discussed earlier (page 1777), evidence for a red cell defect similar to that noted in patients with paroxysmal nocturnal hemoglobinuria has been reported.<sup>58</sup> Splenic sequestration of red cells is demonstrable in some patients.<sup>89</sup> Prior to transfusion, serum iron and transferrin levels usually are normal. Disappearance of injected radioactive iron usually is abnormally rapid, but the percentage appearing in red cells is abnormally low, suggesting that ineffective erythropoiesis (Chapter 13) is present (Fig. 57-3).<sup>32,33,68,70,82,89</sup>

In summary, anemia cannot always be ascribed simply to reduced red cell production in a fibrotic marrow. Normal or even increased erythropoiesis may be present in some patients and, in these, anemia may reflect various degrees of increased plasma volume, reduced red cell survival, and ineffective erythropoiesis.

Leukocyte counts are highly variable, being increased in approximately half the patients and decreased in less than 25% at the time of diagnosis.<sup>89</sup> The numbers of eosinophils and basophils are increased in many patients,

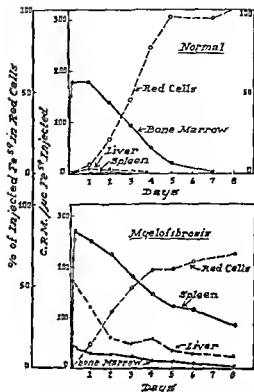


Fig. 57-3 Uptake of  $^{59}\text{Fe}$  into red corpuscles, spleen, liver, and bone marrow in a normal subject and in a patient with myelofibrosis. Iron uptake into red cells is expressed as a percent of the total injected dose and uptake into organs is expressed as externally monitored counts per minute (CPM) in proportion to the number of microcuries injected. (Prepared by Dr. James A. Bush.)

even in those with leukopenia. The number of lymphocytes usually is normal. The marked variation in total leukocyte count ordinarily reflects the variation in neutrophils. However,  $75 \times 10^9$  eosinophils/l were present for a brief time in one of our patients. Immature neutrophils are found in the blood of most patients and a careful search of the blood smear reveals myeloblasts in many. However, as emphasized by Ward and Block,<sup>89</sup> the presence of myeloblasts is not necessarily indicative of conversion to acute leukemia nor is it necessarily a poor prognostic sign. Hypersegmented neutrophils may be seen.

Neutrophil enzymes (Chapter 6) may be abnormal, but extensive surveys have not been reported. Leukocyte alkaline phosphatase

is abnormally high in perhaps two thirds of the patients, low in a few, and normal in the remainder.<sup>59,68,79,84</sup> Peroxidase deficiency of neutrophils was present in one of our patients with IMF. Increased blood histamine, probably secondary to increased histidine decarboxylase in leukocytes,<sup>57</sup> is often present. Increased serum vitamin B<sub>12</sub> levels are found in patients with leukocytosis.<sup>35,39</sup>

Neutrophilia reflects increased neutrophil production, and, when leukocytosis and large numbers of immature neutrophils are present in the blood, kinetic studies reveal a prolonged intravascular survival for neutrophils, similar to that seen in patients with CML.<sup>4</sup> Unlike CML, neutrophil levels in IMF usually do not exhibit a steady increase with time<sup>89</sup> (Fig. 57-4). When extreme leukocytosis is present, differentiation from so-called atypical forms of CML may be difficult (page 1785).

**Platelets** are increased at the time of diagnosis in perhaps 50% of patients with IMF, but, as the disease progresses, thrombocytopenia becomes increasingly common. Extreme degrees of thrombocytosis may be the major feature of the disease in some patients. Abnormally large platelets are present in most and intact or fragmented megakaryo-

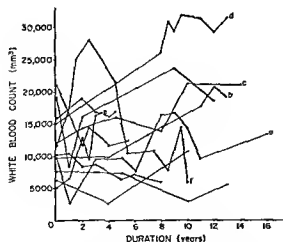


Fig 57-4. Changes in blood leukocyte concentration with time during the natural course of 12 patients with IMF (From Ward and Block,<sup>89</sup> courtesy of the authors and Williams & Wilkins Company)



Fig 57-5. Diffuse fibrosis of the sternal marrow in a patient with myelofibrosis. The nuclei are those of fibroblasts, not of blood cells.

cytes can be found in the blood smears of many.<sup>10,28,55,61,90</sup> Evidence of abnormal platelet function, such as abnormal bleeding time and clot retraction, reduced platelet factor 3, and reduced platelet adhesiveness may be present, even when the platelet count is normal<sup>27,52</sup> (Chapter 35). Failure to release platelet factor 3 was demonstrated in one patient, suggesting that a platelet membrane defect rather than an actual deficiency of the factor was responsible for abnormal platelet function.<sup>63</sup> An abnormally large proportion of platelets is sequestered in the spleen and reduced platelet survival can be demonstrated in some patients.<sup>89</sup>

**BONE MARROW.** Aspiration of bone marrow may prove unsuccessful (dry tap) and in no instance are the results diagnostic of IMF. Smears from successful aspirates may show no abnormality, but more often there is an increased proportion of neutrophil precursors and of megakaryocytes. Marrow biopsy is required to disclose fibrosis. Various degrees of fibrosis are found in almost all patients (Fig. 57-5, Plate III). Standards for defining the normal range in relation to the amount of reticulum fibers present in bone marrow have been suggested.<sup>7</sup> If fibrosis is not found

in the biopsy specimen from a patient suspected of having IMF, a second specimen should be obtained from a different site since fibrosis is not of uniform degree throughout the marrow. Despite some degree of fibrosis, a hyperplastic marrow biopsy is not uncommon in IMF.<sup>89</sup>

**SERUM.** *Uric acid* is increased in the majority of patients,<sup>44, 68, 89</sup> suggesting that a significant increase in the total turnover rate of hematopoietic tissue is occurring. As noted previously (page 1779), gouty arthritis and renal stones may complicate the disease but tophi are unusual, as is urate nephropathy (Chapter 54).

Serum *lactic dehydrogenase* is elevated in most patients.<sup>56, 89</sup> Serum *alkaline phosphatase* is increased in perhaps half, but this probably reflects disease of bone rather than of the liver.<sup>89</sup>

## Pathology

The characteristic features are fibrosis of bone marrow (page 1781), osteosclerosis, and extramedullary hematopoiesis.

**Osteosclerosis**, severe enough to be detected on x-ray examination (Fig. 57-6), has been reported in from 30 to 70% of the patients.<sup>89</sup> The axial skeleton and proximal portions of the long bones are affected most commonly. The bony cortex appears thickened and the normal trabecular pattern is lost. In patients with advanced cases the marrow cavity may be obliterated. On section, the bony trabeculae are abnormally thick, irregular, and twisted. New bone formation occurs around existing trabeculae and represents ossification of wavy, argyrophilic fibers after a dense network of such fibers has formed.<sup>73</sup> Blood flow to bone is increased.<sup>91</sup>

**Extramedullary hematopoiesis** is most commonly (almost invariably) present in the spleen and is believed to be solely responsible for the splenic enlargement. The liver likewise is involved in most patients. Virtually any organ may be engaged in extramedullary hematopoiesis and in approximate decreasing frequency this is found in lymph nodes, kidneys, adrenals, peritoneum, gut, pleura, lungs,

fatty tissue, skin, breast, dura, ovaries, and thymus gland.<sup>89</sup> The hematopoietic islands may contain only one type of blood cell precursor, or erythropoiesis, granulocytopenia, and megakaryocytopenia may all be found in the same area. There may be scattered, small foci of but a few cells each, or large, macroscopic tumors of hematopoietic tissue, like those in certain other disorders may be present<sup>33a</sup> (Fig. 57-7). Except for compression of adjacent normal tissue, the organs involved apparently are not damaged and their normal architecture usually is preserved.

Extramedullary hematopoiesis can be demonstrated by needle biopsy or by aspiration of the spleen or needle biopsy of the liver.<sup>89</sup> Neither of these procedures is without risk, however, particularly in patients who may have a tendency to bleed.<sup>89</sup>

## Complications and Cause of Death

Infection is the leading cause of death followed by congestive heart failure, renal failure, hepatic failure, bleeding, and thrombosis.<sup>12, 42, 53, 59, 68, 71, 81, 89</sup>

Weight loss often becomes a troublesome feature of IMF, particularly in the advanced stages of the disease. While it often is accompanied by anorexia, it usually appears to be out of proportion to the degree of anorexia. Edema of the lower extremities is common and, in most subjects, no specific cause is found. It may possibly be produced by the enlarged spleen and liver with compression of the inferior vena cava.

Infection, most commonly pneumonia, in most patients cannot be attributed to either immune deficiency or to neutropenia. Co-existing serious illnesses such as congestive heart failure or chronic lung disease usually are also present when death is associated with infection.

Portal hypertension with esophageal varices complicates the course of 10%<sup>68</sup> to 17%<sup>89</sup> of patients with IMF. It may be due to unrelated causes, such as alcoholism in some patients, but also has been produced by thrombosis of hepatic veins (Budd-Chiari syndrome<sup>89</sup>), compression<sup>33a</sup> or thrombosis

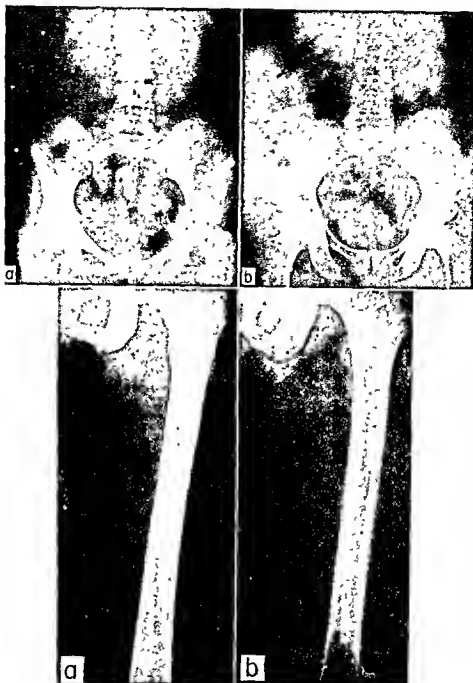


Fig. 57-6. Roentgenograms of the spine, hip bones, and femur of a woman with myelofibrosis (a), compared with those of a normal woman of the same age (b). Note especially the striking contrast between the density of the vertebrae and of the spongiosa of the long bones in the two subjects.

of the portal vein,<sup>29,74,78</sup> transfusion-induced hemochromatosis, and possibly intrasinusoidal hematopoiesis.

Bleeding may reflect thrombocytopenia or functionally defective platelets (page 1781).

Thrombosis usually is associated with thrombocytosis, but is in no sense common.

Amyloidosis producing the nephrotic syndrome<sup>67</sup> proved fatal in one of our patients. Rarely, tumors composed of hematopoietic

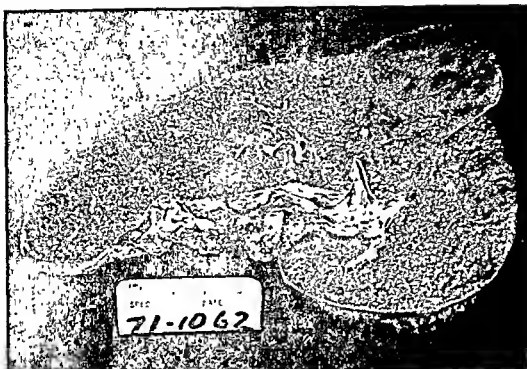


Fig 57-7 A large nodule of extramedullary hematopoiesis in the spleen of a patient with Gaucher's disease. Microscopic examination revealed the nodule to be composed of erythroid tissue primarily (Courtesy of R E Lee MD)

tissue may compress vital structures such as the spinal cord.<sup>21,33a,48</sup>

The frequency of severe, chronic pain in the bones and spleen has been emphasized by some authors,<sup>37</sup> but we have not been impressed that these are common complications in IMF, either from our own experience or from review of the literature.

*Splenic rupture* has occurred spontaneously but is exceedingly rare<sup>89</sup> and has also been reported following needle biopsy of the spleen.<sup>89</sup> *Splenic infarction* may occur but is also rare and requires no treatment other than administration of analgesics (Chapter 48).

### Therapy

Treatment should be directed toward specific complications but no form of therapy has any proven beneficial effect upon the basic pathologic process. Antitumor therapy, as advocated by some,<sup>12,23,79</sup> is as likely, or more likely, to be harmful than helpful. Most patients require no form of therapy.

Busulfan reduces spleen size and lowers the

leukocyte and platelet counts but rarely relieves the anemia.<sup>12,15,25,55,69,79</sup> Other agents such as <sup>32</sup>P and TEM<sup>89</sup> have similar effects. The risk of thrombosis because of thrombocytosis is small and rarely justifies an attempt to reduce platelet production. While serious thrombotic and thromboembolic disease may be observed in association with thrombocytosis, proof that the latter is responsible for the clotting disorder is lacking. For this reason, we do not consider any form of myelo-suppressive therapy to be useful or justified in patients with IMF. In any event, if anti-tumor therapy is used, the agents must be given in doses less than those used in CML as severe pancytopenia is often induced in IMF. For example, 2 mg of busulfan per day probably comprise the maximal dose that can be given with any degree of safety to patients with IMF.

*Splenectomy* has been advocated early in the disease in virtually all patients with IMF,<sup>23</sup> but the evidence to support such a recommendation is unconvincing. Splenectomy, however, can be considered in two

circumstances. On rare occasions, life-threatening degrees of thrombocytopenia are due primarily to pooling and destruction of platelets by the spleen.<sup>89</sup> Occasionally, severe anemia requiring frequent transfusion is due primarily to splenic red cell sequestration and destruction. In either instance, a shortened intravascular life span of platelets and/or red cells as well as their localization in the spleen should be demonstrable by kinetic studies before splenectomy is considered. Irradiating the spleen<sup>43,49,59,68,89</sup> reduces its size but has little effect on splenic destruction of cells.

At one time, splenectomy was thought to be always contraindicated in IMF.<sup>10,11,42,49,72</sup> primarily because of the very high, immediate postoperative mortality. Additional experience has proved this view not to be so well established.<sup>18,89</sup> However, rapid hepatic enlargement may follow splenectomy and increased destruction of platelets and red cells may take place in the liver.<sup>13,14,36</sup> Extreme thrombocytosis may follow splenectomy in IMF; counts as high as  $8,000 \times 10^9/l$  have been reported.<sup>12,15</sup>

**Androgen** therapy may be used as treatment for severe anemia, especially when anemia is due primarily to decreased red cell production.<sup>32</sup> The synthetic androgen, oxymetholone, is probably more effective and has fewer side effects than native testosterone and has the added advantage of oral administration. Oxymetholone is given in a daily dose of 2 to 4 mg/kg. Benefit is most often observed in relatively young women with minimal splenomegaly.<sup>32,53,79</sup> If no improvement is noted after three to six months the therapy should be discontinued. *Adrenal steroids* have no proven beneficial effects in IMF.

**Allopurinol** should be used to keep serum uric acid within normal limits in order to avoid urate nephropathy and renal calculi and to reduce the frequency of gout (Chapter 54).

## Survival

As with any disease in which a very long asymptomatic period often precedes the diagnosis, data for survival from the time of diagnosis are quite variable. Survival for many decades is possible<sup>89</sup> and median sur-

vival has ranged from one to five years in various series (Table 57-1). In general, the more striking the evidence of disease at diagnosis, the shorter is survival likely to be.<sup>89</sup>

Occasionally, severe pancytopenia develops very rapidly with death ensuing shortly from bleeding and/or infection, a circumstance that has been termed "acute"<sup>78</sup> or "malignant"<sup>61</sup> myelofibrosis. In such cases, pancytopenia seems to reflect reduced cell production rather than splenic destruction.

**Table 57-1. Median Survival in Idiopathic Myelofibrosis\***

Author <sup>a</sup>	Number of Patients	Median Survival from Diagnosis (Years)
Koler <sup>24</sup>		
Summation of literature	119	4.0
Own series	24	4.2
Rosenthal and Maloney <sup>74a</sup>	98	1.4
Silverstein et al <sup>34</sup>		
Symptomatic patients	89	4.5
Asymptomatic	48	5.0
Pitcock et al <sup>71</sup>	52	1.4
Ward and Block <sup>89</sup>	46	5.2

\*Adapted from Table 13 of Ward and Block.<sup>89</sup>

## Diagnosis and Differential Diagnosis

The possibility of secondary forms of myelofibrosis and extramedullary hematopoiesis (page 1786) must be excluded by appropriate studies and IMF must be distinguished from CML, AML, and polycythemia rubra vera (PRV). Whether one should distinguish between IMF, "atypical" CML (Chapter 48), and "idiopathic thrombocytopenia" (Chapter 34) or consider these syndromes as variants of IMF is uncertain (Chapter 48).

Termination of PRV in an IMF-like syndrome occurs (Chapter 30) and marrow fibrosis may be present in CML and in AML (Chapters 47 and 48). Whether IMF ever "converts" to either AML or CML may be questioned. It is impossible at present to be

certain that a patient with typical IMF has not had "typical" but undiagnosed PRV in the past. Similarly there is no means of determining whether a patient with polycythemia, fibrosis of marrow, and extramedullary hematopoiesis should be properly designated as having IMF or PRV.

In most cases, separation of IMF and CML is relatively easy. The single, most reliable diagnostic test is cytogenetic analysis. Approximately 90% of patients with typical CML have the Philadelphia (Ph<sup>1</sup>) chromosome abnormality (Chapter 48). Only two patients,<sup>30,65</sup> and perhaps a third,<sup>16</sup> who were thought to have IMF were reported to have a Ph<sup>1</sup> chromosome out of more than 200 patients in whom cytogenetic studies were done.<sup>69</sup> However, it was not demonstrated that the Ph<sup>1</sup>-like defect was indeed chromosome number 22 and in that regard it may be significant that reexamination<sup>69</sup> of two patients with PRV reported to have a Ph<sup>1</sup> defect demonstrated it to be an abnormal X chromosome rather than number 22. If the Ph<sup>1</sup> abnormality is absent, difficulties may be encountered in distinguishing CML from IMF. As discussed in Chapter 48, patients thought to have CML who do not have the Ph<sup>1</sup> defect often have atypical features and respond poorly to busulfan, resembling IMF in that regard. Pathologic examination may not be helpful in distinguishing such cases from those of IMF.<sup>19</sup> However, in patients with typical CML the size of the spleen is directly proportional to the white count. Thus, if the spleen is very large but the leukocyte count is less than  $100.0 \times 10^9/l$ , one must suspect that IMF rather than CML is present. The leukocyte alkaline phosphatase is not particularly helpful, for, while it is low in most patients with CML and high in most of those with IMF, there are patients with typical CML who have a high LAP (Chapter 48) and patients with IMF who have a low LAP (page 1780). Perhaps all patients with atypical CML (Chapter 48) should be considered as having a disease more closely allied to IMF than to CML.

There are numerous reports of patients with IMF who have developed AML.<sup>12,68,71,80,81</sup> However, in most the di-

agnosis of AML rested simply on the presence of myeloblasts in the blood and, as discussed earlier (page 1780), this is a common finding in IMF. Unless there is evidence for a steadily increasing number of myeloblasts in marrow, blood, or other organs as well as a steadily decreasing number of normal blood cells the diagnosis of IMF converting to AML probably should not be entertained. The development of previously absent chromosome defects also has been suggested as evidence for true leukemic conversion.<sup>50</sup>

Idiopathic thrombocytopenia (Chapter 34) may be closely related to IMF and, indeed, whether it should be considered a distinct syndrome can be questioned.<sup>5,63,89</sup>

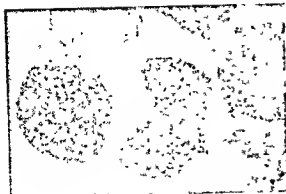
## Secondary Forms of Myelofibrosis

Syndromes very similar to IMF have been observed in association with a variety of neoplasms, infections, and other diseases, as well as following toxic exposure to chemicals such as benzol (Table 57-2). Blood changes similar to those observed in IMF (page 1779) with or without fibrosis and extramedullary hematopoiesis have been described. Particularly when nucleated red cells and myeloid precursors are prominent in the blood the general term *myelophthisic anemia* or *leukoerythroblastosis* has been used. These terms do not refer to specific entities.

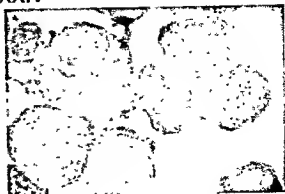
The mechanism by which secondary MF or leukoerythroblastosis is produced is as poorly understood as is the cause of IMF (page 1777). Tumor has not always been demonstrable in the bone marrow when MF developed, and the MF has been reversed when therapy of the primary disease led to overall improvement, as in Hodgkin's disease<sup>47</sup> or carcinoma of the prostate.<sup>28a</sup>

The diagnosis of secondary MF is based on discovering an associated disease or exposure to a toxic agent. A history of excessive exposure to benzol makes this a presumptive cause. When MF is secondary to infection, the infection usually is chronic, widespread, and easily diagnosed. With the availability of treatment for tuberculosis and the chronic fungal infections, MF secondary to infection

# PLATE XXIV



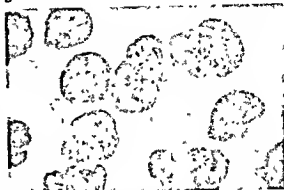
A



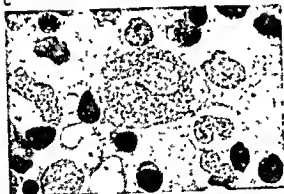
B



C



D



E



F



G

Tumor cells and other unusual cells from bone marrow and blood (Wright's stain,  $\times 1000$ ) A, Carcinoma of the lung, B, carcinoma of the breast, C, carcinoma of the colon, D, neuroblastoma, E, Reed Sternberg-like cell, F, Reed Sternberg-like cell, G, tumor cells and other unusual cells from the lining of a vein



**Table 57-2. Some Conditions Associated with Myelofibrosis and/or Leukoerythroblastosis<sup>1,2,4,22,29,87,88</sup>**

**Tumors**

**Leukemias**

Chronic myelocytic leukemia

Acute myeloblastic leukemia

Multiple myeloma

Hodgkin's disease

Lymphosarcoma

Reticulum cell sarcoma

Carcinoma, breast

prostate

stomach

etc

**Infection**

Tuberculosis

Osteomyelitis (focal fibrosis)

**Other diseases**

Polycythemia rubra vera

Marble bone disease

Paget's disease (focal fibrosis)

Gaucher's disease

Amlyoidosis

Xanthomatosis

**Toxic exposure**

X-irradiation

Benzol

Fluorine

**Produced in experimental animals with**

Strontium

Irradiation

Phosphorus

Estrogens

Saponin

etc

has become quite unusual.<sup>3</sup> In most cases associated with tumor, the tumor has been widely metastatic and is thus easily recognized.

Therapy consists of treating the underlying disease or avoiding toxic exposure.

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# Appendices

# APPENDIX A: I. Red Cell Values in Normal Adults

**Table A-1. Measures of Red Cell Concentration in Normal Adults at Sea Level**

Determination	Method	Males		Females	
		Mean	95% Range	Mean	95% Range
Erythrocytes ( $\times 10^{12}/l$ )	Hemocytometer <sup>48</sup>	5.4	4.5-6.3	4.8	4.2-5.5
	Electronic <sup>29</sup>	5.2	4.4-6.0		
Hemoglobin (g/dl)	Cyanmethemoglobin <sup>48</sup>	16.0	14.0-18.0	14.0	12.0-16.0
VPRC (l/l)	Macro <sup>48</sup>	0.47	0.40-0.54	0.42	0.37-0.47
	Micro <sup>20</sup>	0.46	0.41-0.51		
Reticulocytes <sup>15,27</sup> (per cent) ( $\times 10^9/l$ )	New methylene blue	1.8	0.8-2.5	1.7	0.8-4.1
		88	18-158		
Sedimentation rate <sup>9</sup> (mm/hr) (mm/hr) (ml/dl)	Wintrobe or				
	Westergren	4	0-10	10	0-20
	Zeta sedimentation rate		40-51		40-51

**Table A-2. Erythrocyte Indices in Normal Adults**

	Method	Mean	95% Limits
MCV (fl)	RBC Hemocytometer		
	VPRC Macro <sup>48</sup>	87	80-94
	RBC Electronic		
	VPRC Micro <sup>9</sup>	90	83-97
	RBC Hemocytometer		
	VPRC Micro <sup>20</sup>	85	77-93
	RBC Electronic		
	VPRC Micro <sup>20</sup>	88	80-96
	Coulter model S <sup>13</sup>	91	82-101
	RBC Hemocytometer <sup>48</sup>	29	26-32
MCH (pg/cell)	Coulter model S <sup>13</sup>	31	27-34
	VPRC Macro	34	32-36
MCHC (g/dl RBC's)	VPRC Micro <sup>20</sup>	33	31-35
	Coulter model S <sup>13</sup>	34	31.5-36

**Table A-3. Effect of Altitude on VPRC in Normal Males<sup>9</sup>**

Altitude		n	VPRC (l/l)*
ft	meters		
0	0	721	0.47
4,400	1340	744	0.495
7,457	2280	100	0.51
12,240	3740	40	0.54
14,800	4540	32	0.61
17,800	5430	10	0.69

\*Mean values in males

## APPENDIX A: II. Leukocyte Values in Normal Adults

**Table A-10. Leukocytes. Absolute and Relative Values for a Normal Population (291 Adults in Salt Lake City)**

	Absolute Number, Cells $\times 10^3/l$ from log plots		Relative Number, %	
	Median	95% range	Mean	95% range
Total leukocytes	7.0	4.3-10.0		
Neutrophils—"bands"	0.52	0.1-2.1	9.5	0-21.5
Neutrophils—"segs"	3.0	1.1-6.05	43.5	24.8-62.3
Neutrophils—total	3.65	1.83-7.25	53.0	34.6-71.4
Eosinophils	0.15	0-0.7	3.2	0-7.8
Basophils	0.03	0-0.15	0.6	0-1.8
Lymphocytes	2.6	1.5-4.0	36.1	19.6-52.7
Monocytes	0.43	0.2-0.95	7.1	2.4-11.8

From Orfanakis et al.<sup>34</sup> courtesy of the authors and Williams and Wilkins Company

**Table A-11. Scoring Criteria for Leukocyte Alkaline Phosphatase**

Cell Rating	Amount	Precipitated Azo Dye in Cytoplasm		
		Granule Size	Staining Intensity	Background of Cytoplasm
0	0	None		No staining
1+	50%	Small	Faint to moderate	Colorless to very pale pink
2+	40-60%	Small to medium	Moderate to strong	Colorless to pale pink
3+	80-100%	Medium to large	Strong	Colorless to pink
4+	100%	Medium and large	Brilliant	Not visible

\*Percentage of volume of cytoplasm occupied by azo dye precipitate  
From Kaplow,<sup>34</sup> courtesy of the author and Williams & Wilkins Company

**Table A-12. Leukocyte Alkaline Phosphatase Scores in Normal Subjects**

Group	Number	Mean	95% Limits
Male	51	73	22-124
Female	50	91	33-149
Total	101	82	25-139

From Cartwright\* courtesy of the author and Grune & Stratton

## APPENDIX A: III. Tests of Hemostasis and Blood Coagulation

**Table A-13. Normal Values for Tests of Hemostasis and Blood Coagulation\***

<i>Test</i>	<i>Normal Range (<math>\pm 2</math> SD)</i>
Platelet count	140–440 $\times 10^9/l$
Bleeding time	
Ivy	1–9 min†
Duke	1–4 min
Partial thromboplastin time	
Standard	68–82 sec‡
Activated	32–46 sec‡
Plasma prothrombin time	11–15 sec§
Coagulation time	
Glass tubes	8–18 min
Plasma thrombin time	13–17 sec
Fibrinogen assay	160–415 mg/dl
Euglobulin clot lysis time	>2 hours‡
Fibrin degradation products	>8 $\mu g/dl$ ‡
Specific assays for individual coagulation factors	50–200% of normal

\*References to methods employed are included in Table 33-2, page 1050

† Confidence limits determined by logarithmic plot

‡ Minor variations depend on exact technique employed

§ Significant variations depend on thromboplastin used

# APPENDIX A: IV. Bone Marrow in Normal Adults

Table A-14. Differential Counts of Bone Marrow Aspirates from 12 Healthy Men

	Mean (%)	Observed Range (%)	95% Confidence Limits (%)
<b>NEUTROPHILIC SERIES (total)</b>	<b>53.6</b>	<b>49.2-65.0</b>	<b>33.6-73.6</b>
Myeloblasts	0.9	0.2-1.5	0.1-1.7
Promyelocytes	3.3	2.1-4.1	1.9-4.7
Myelocytes	12.7	8.2-15.7	8.5-16.9
Metamyelocytes	15.9	9.6-24.6	7.1-24.7
Band	12.4	9.5-15.3	9.4-15.4
Segmented	7.4	6.0-12.0	3.8-11.0
<b>EOSINOPHILIC SERIES (total)</b>	<b>3.1</b>	<b>1.2-5.3</b>	<b>1.1-5.2</b>
Myelocytes	0.8	0.2-1.3	0.2-1.4
Metamyelocytes	1.2	0.4-2.2	0.2-2.2
Band	0.9	0.2-2.4	0-2.7
Segmented	0.5	0-1.3	0-1.1
<b>BASOPHILS AND MAST CELLS</b>	<b>&lt;0.1</b>	<b>0-0.2</b>	
<b>ERYTHROCYTIC SERIES (total)</b>	<b>25.6</b>	<b>18.4-33.8</b>	<b>15.0-36.2</b>
Pronormoblasts	0.6	0.2-1.3	0.1-1.1
Basophilic	1.4	0.5-2.4	0.4-2.4
Polychromatophilic	21.6	17.9-29.2	13.1-30.1
Orthochromatic	2.0	0.4-4.6	0.3-3.7
Lymphocytes	16.2	11.1-23.2	8.6-23.8
Plasma cells	1.3	0.4-3.9	0-3.5
Monocytes	0.3	0-0.8	0-0.6
Megakaryocytes	<0.1	0-0.4	
Reticulum cells	0.3	0-0.9	0-0.8
M:E ratio	2.3	1.5-3.3	1.1-3.5

# APPENDIX A: *V. Blood and Bone Marrow Values in Normal Embryo, Infants, Children, and Adolescents*

**Table A-15. Normal Values for Red Corpuscles at Various Ages**

	Red Cell Count ( $\times 10^{12}/l$ )	Hemoglobin (g/dl)	Vol Packed RBC (l/l)	Corpuscular Values			
				MCV (fl)	MCH (pg/cell)	MCHC (g/dl RBC's)	MCD ( $\mu m$ )
First day	$5.1 \pm 1.0$	$19.5 \pm 5.0$	$0.54 \pm 0.10$	106	38	36	8.6
2-3 days	5.1	19.0	0.53	105	37	35	
4-8 days	5.1	$18.3 \pm 4.0$	0.52	103	36	35	
9-13 days	5.0	16.5	0.49	98	33	34	
14-60 days	$4.7 \pm 0.9$	$14.0 \pm 3.3$	$0.42 \pm 0.07$	90	30	33	8.1
3-5 months	$4.5 \pm 0.7$	$12.2 \pm 2.3$	0.36	80	27	34	7.7
6-11 months	4.6	11.8	$0.35 \pm 0.05$	77	26	33	7.4
1 year	4.5	11.2	0.35	78	25	32	7.3
2 years	4.6	11.5	0.35	77	25	32	
3 years	4.5	12.5	0.36	80	27	35	7.4
4 years	$4.6 \pm 0.6$	12.6	0.37	80	27	34	
6 years	4.6	12.6	0.37	80	27	34	
6-10 years	4.7	12.9	0.37	80	27	34	7.4
11-15 years	4.8	13.4	0.39	82	28	34	



Table A-16. Hematologic Values of the Human Embryo

Age (in weeks)	Hb (g/dl)	Hematocrit (l/l)	RBC ( $10^{12}/l$ )	MCV (fl)	MCH (pg/red cell)	MCHC (g/dl RBC)	Nuc RBC (% of RBC)	Ratio (%)	Diam ( $\mu$ m)	Granulocytes ( $10^9/l$ )	Lymphocytes ( $10^9/l$ )
12	8.0-10.0	0.33	1.5	180	60	34	5.0-8.0	40	10.5		
16	10.0	0.35	2.0	140	45	33	2.0-4.0	10-25	8.5		
20	11.0	0.37	2.5	135	44	33	1.0	10-20	9.0	1.0	5.0
24	14.0	0.40	3.5	123	38	31	1.0		8.8	1.5	
28	14.5	0.45	4.0	120	40	31	0.5		8.7		
34	15.0	0.47	4.4	118	38	32	0.2		8.5	2.0	2.5

From Oski and Naimen,<sup>35</sup> slightly modified. Courtesy of the authors and W.B. Saunders Company

**Table A-17. Normal Hematologic Values during the First Month of Life in the Term Infant**

<i>Value</i>	<i>Cord Blood</i>	<i>Day 1</i>	<i>Day 3</i>	<i>Day 7</i>	<i>Day 14</i>	<i>Day 28</i>
Hb (g/dl)	16.8	18.4	17.8	17.0	16.8	15.6
Hematocrit (l/l)	0.53	0.58	0.55	0.54	0.52	0.45
Red cells ( $\times 10^{12}/l$ )	5.25	5.8	5.6	5.2	5.1	4.7
MCV (fl)	107	108	99	98	96	91
MCH (pg/red cell)	34.0	35.0	33.0	32.5	31.5	31.0
MCHC (g/dl RBC's)	31.7	32.5	33.0	33.0	33.0	32.0
Reticulocytes (%)	3-7	3-7	1-3	0-1	0-1	0-1
Nucleated RBC ( $\times 10^9/l$ )	500	200	0-5	0	0	0
Platelets ( $\times 10^9/l$ )	290	192	213	248	252	240

*From Oski and Naiman,<sup>15</sup> slightly modified. Courtesy of the authors and W B Saunders Company*

Table A-18. White Blood Cell and Differential Counts during the First Two Weeks of Life

Age	Leukocytes	Neutrophils			Eosinophils	Basophils	Lymphocytes	Monocytes
		Total	Seg	Band				
Birth								
Mean ( $\times 10^9/l$ )	18.1	11.1	9.4	1.6	0.4	0.1	5.5	1.05
Range ( $\times 10^9/l$ )	9.0-30.0	6.0-28			0.02-0.85	0-0.64	2.0-11.0	0.4-3.1
Mean (%)	—	61	52	9	2.2	0.5	31	5.8
7 Days								
Mean ( $\times 10^9/l$ )	12.2	5.5	4.7	0.83	0.5	0.05	5.0	1.1
Range ( $\times 10^9/l$ )	5.0-21.0	1.5-10.0			0.07-1.10	0-0.25	2.0-17.0	0.3-2.7
Mean (%)	—	45	39	6	4.1	0.4	41	9.1
14 Days								
Mean ( $\times 10^9/l$ )	11.4	4.5	3.9	0.63	0.35	0.05	5.5	1.0
Range ( $\times 10^9/l$ )	5.0-20.0	1.0-8.5			0.07-1.0	0-0.23	2.0-17.0	0.2-2.4
Mean (%)	—	40	34	5.5	3.1	0.4	48	8.8

From Oski and Naiman,<sup>15</sup> courtesy of the authors and WB Saunders Company

**Table A-19. The Bone Marrow Differential during the First Week of Life**

	<i>0-24 Hours (%)</i>	<i>7 Days (%)</i>	<i>Adult</i>
Myeloblasts	0-2	0-3	0.3-5.0
Promyelocytes	0.5-6.0	0.5-7.0	1.0-8.0
Myelocytes	1.0-9.0	1.0-11.0	5.5-22.5
Metamyelocytes	4.5-25.0	7.0-35.0	13.0-32.0
Band forms	10.0-40.0	11.0-45.0	—
Erythroblasts	0-1.0	0-0.5	1.0-8.0
Proerythroblasts	0.5-9.0	0-0.5	2.0-10.0
Normoblasts	18.0-41.0	0-15.0	7.0-32.0
Myeloid: erythroid ratio	1.5:1.0	6.5:1.0	3.5:1.0

From Oski and Naiman,<sup>35</sup> courtesy of the authors and WB Saunders Company

**Table A-20. Changes in Differential Counts of Bone Marrow with Age\***

		<i>Birth</i>	<i>1 Week</i>	<i>1 Week to 1 Year</i>	<i>1-4 Years</i>	<i>4-12 Years</i>	<i>Adult</i>
Neutrophilic series	$\bar{x}$ %	54	65	37	50	52	57
	95 % limits	31-77	21-79	22-52	32-69	35-69	39-79
Eosinophilic series	$\bar{x}$ %	3	3	3	6	3	3
	95 % limits	1-5	1-5	1-5	2-10	1-5	1-5
Lymphocytes	$\bar{x}$ %	6	13	36	22	18	17
	95 % limits	2-10	7-19	18-54	8-36	12-28	10-24
Erythrocytic	$\bar{x}$ %	34	15	17	19	21	20
	95 % limits	18-50	5-25	7-27	11-27	11-31	10-30
M:E ratio	$\bar{x}$	1.6	4.3	2.2	2.6	2.5	2.8

\*For references regarding source see Table 2.2, page 71

# APPENDIX A: VI. Values for Various Other Measurements in Normal Subjects

**Table A-21. Iron and Copper in Plasma**

Measurement	Mean	Lower Limit of Normal	Upper Limit of Normal
Plasma iron			
μg/dl	♂ 122 ♀ 109	71 (43-112) 62 (28-101)	201 (112-276) 173 (130-202)
μmol/l	♂ 21.8 ♀ 19.5	12.7 (7.7-20.0) 11.1 (5.0-18.0)	35.9 (20.0-49.3) 30.9 (23.2-36.1)
Plasma total iron binding capacity			
μg/dl	340	253 (224-306)	435 (429-472)
μmol/l	60.7	45.2 (40.0-54.6)	77.7 (76.6-84.3)
Transferrin saturation (%)	35	20	50
Plasma copper			
μg/dl	114	81	147
μmol/l	18.1	13	23

\*Range of values from several laboratories<sup>3,6,10,23,26,30,31,37,38,40,42,44,45</sup> given in parentheses

**Table A-22. Serum Folate, Vitamin B<sub>12</sub>, and Related Determinations<sup>11</sup>**

Measurement	Mean	Lower Limit of Normal	Upper Limit of Normal
Serum vitamin B <sub>12</sub> (ng/l)	450	160 (100-270)	1000 (750-1200)
Serum folate (μg/l)	8.1	3 (2.1-7.5)	25 (9-28)
Erythrocyte folate (μg/l)	274	100 (50-325)	600 (300-875)
Urinary methylmalonate (mg/day)	—	—	9
Urinary formiminoglutamate (FIGlu) (mg/day)	—	1.0	17

\*Range of values from several laboratories given in parentheses

**Table A-23. Vitamin B<sub>12</sub> Absorption by the Urinary Excretion Method (Schilling Test)<sup>11</sup>**

Oral Vitamin B <sub>12</sub> Dose (μg)	Urinary Vitamin B <sub>12</sub> Excretion (%)	
	24 hr*	48 hr*
0.5	28 (16-40)	37 (21-48)
1.0	22 (11-39)	29 (14-45)
2.0	11 (5-17)	17 (11-34)

\*With 24-hour collections, one injection of 1000 μg "cold" B<sub>12</sub> was administered, with 48-hour collections, two injections of 1000 μg "cold" B<sub>12</sub> were administered at 24-hour intervals

Table A-24. Concentrations of Immunoglobulins in Serum of Normal Subjects at Different Ages

Age	No of Subjects	Level of $\gamma G$			Level of $\gamma M$			Level of $\gamma A$			Level of Total $\gamma$ -Globulin*		
		mg/dl (range)	% of adult level		mg/dl (range)	% of adult level		mg/dl (range)	% of adult level		mg/dl (range)	% of adult level	
Newborn	22	1,031 $\pm$ 200 (645-1,244)	99 $\pm$ 17		11 $\pm$ 5 (5-30)	11 $\pm$ 5		2 $\pm$ 3 (0-11)	1 $\pm$ 2		1,044 $\pm$ 201 (660-1,439)	67 $\pm$ 13	
1-3 mo	29	430 $\pm$ 119 (272-762)	37 $\pm$ 10		30 $\pm$ 11 (16-67)	30 $\pm$ 11		21 $\pm$ 13 (6-66)	11 $\pm$ 7		481 $\pm$ 127 (324-699)	31 $\pm$ 9	
4-6 mo	33	427 $\pm$ 199 (205-1,125)	37 $\pm$ 16		43 $\pm$ 17 (10-63)	43 $\pm$ 17		29 $\pm$ 16 (9-93)	14 $\pm$ 9		498 $\pm$ 204 (229-1,232)	32 $\pm$ 13	
7-12 mo	56	661 $\pm$ 219 (279-1,533)	58 $\pm$ 19		54 $\pm$ 23 (22-147)	55 $\pm$ 23		37 $\pm$ 16 (19-98)	19 $\pm$ 9		752 $\pm$ 242 (327-1,997)	48 $\pm$ 16	
13-24 mo	59	762 $\pm$ 209 (259-1,393)	68 $\pm$ 16		56 $\pm$ 23 (14-114)	59 $\pm$ 23		60 $\pm$ 24 (19-119)	25 $\pm$ 12		670 $\pm$ 269 (399-1,598)	69 $\pm$ 19	
25-39 mo	33	992 $\pm$ 193 (419-1,274)	77 $\pm$ 16		61 $\pm$ 19 (26-113)	62 $\pm$ 19		71 $\pm$ 37 (19-235)	36 $\pm$ 19		1,024 $\pm$ 205 (499-1,416)	65 $\pm$ 14	
3-6 yr	29	928 $\pm$ 226 (569-1,597)	90 $\pm$ 20		56 $\pm$ 16 (22-100)	67 $\pm$ 16		93 $\pm$ 27 (55-152)	47 $\pm$ 14		1,076 $\pm$ 246 (730-1,771)	69 $\pm$ 17	
6-9 yr	19	923 $\pm$ 266 (559-1,492)	90 $\pm$ 22		65 $\pm$ 25 (27-116)	66 $\pm$ 25		124 $\pm$ 45 (54-221)	62 $\pm$ 23		1,112 $\pm$ 293 (640-1,725)	71 $\pm$ 20	
9-11 yr	9	1,124 $\pm$ 235 (779-1,456)	97 $\pm$ 20		79 $\pm$ 33 (35-132)	80 $\pm$ 33		131 $\pm$ 60 (12-208)	66 $\pm$ 30		1,334 $\pm$ 254 (966-1,639)	95 $\pm$ 17	
12-16 yr	9	946 $\pm$ 124 (726-1,095)	62 $\pm$ 11		59 $\pm$ 20 (35-72)	60 $\pm$ 20		146 $\pm$ 63 (70-229)	74 $\pm$ 32		1,153 $\pm$ 169 (933-1,294)	74 $\pm$ 12	
Adults	30	1,159 $\pm$ 305 (569-1,919)	100 $\pm$ 26		99 $\pm$ 27 (47-147)	100 $\pm$ 27		200 $\pm$ 61 (61-330)	100 $\pm$ 31		1,457 $\pm$ 353 (730-2,365)	100 $\pm$ 24	

\*Mean  $\pm$  1 SD

For additional data see references 1 and 21

From Stehm and Fudenberg,<sup>41</sup> courtesy of the authors and Pediatrics

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## APPENDIX B: *Comparative Hematology—Tabulated Data and Bibliography*

In Tables B-1 and B-2 are listed blood counts as recorded by a number of different observers in a large variety of animals. The tabulated data are representative but not exhaustive. Some blood counts are not given in the tables but will be found in the references cited, especially in the monographs on comparative hematology that are listed. Descriptions of cells and excellent illustrations will be found in the articles cited. Many noteworthy studies have been made concerning the hemoglobins of various animals and these are cited.

Leukocyte counts, both total and differential, fluctuate much more in animals than in man in the absence of disease. The data recorded give only average values and a considerable range about these means should be expected.



Table B-1. Blood Counts in Thirty-one Species of Mammals

Species	Author	RBC	Hgb	Hct	MCV	MCH	MCHC	Distn	WBC	N	E	B	L	Ato	Pl	No. of Animals
Baboon	Burns et al. <sup>112</sup>	4.72±0.35	17.77±1.17	40.12±3.44	84-4-7	28.6±1.9	31.8±2.4	7.3±3.8	11.4	81.0	21.0	2.5	9.5	6.0		21
Camel	Ponder et al. <sup>113</sup>	10.62														
Cap	Landisburg <sup>114</sup>	7.24±1.01	10.5±2.1	40.2±8.1	87±6	15.1±2.2	27-4-1	5.9	17.2±6.8	58.3	8.9	0	33.0	0.8	232±62	52
	Scarborough <sup>115</sup>	8.43±1.40							13.8	57.1	5.3	0.1	32.5	5.9		130±
	Vaughan <sup>116</sup>	7.85	12.6						8.2-24.0	42-84	1-18	1.0	6-45	1-3		20
Chimpanzee	Burns et al. <sup>112</sup>	4.68±0.72	12.05±1.66	38.32±8.47	78.2±9.5	24.9±2.5	31.3±2.8		10.13-14.08	83.0	7.0	0	40.0	0		42
	Wintrobe et al. <sup>117</sup>	5.11	12.3	41.8	82	25	30	7.4	12.0-22.0	58.0	5.0	20.0	18.0	1.0	280	10
	Ponder et al. <sup>113</sup>	8.30							10.4							1
Coat munt.	Zundel <sup>118</sup>	8.97	9.1	31.0	45	13	30		b1	65.0	4.0	1.0	26.0	4.0	333	
Cow	Kuhnke <sup>119</sup>	5.8-6.6	(Cows, bulls and calves of different ages)					6.0-5.9								219
	Osthaus <sup>120</sup>	8.70	(in a mixture of ages)						10.7	19.8	3.3	0	64.4	12.2		8
		8.38	(three to six and a half years of age)						10.2	28.8	7.0	0	58.1	6.0		5
	Crastach <sup>121</sup>	8.41	10.6	33.0	62	19	32									1
	Wintrobe <sup>122</sup>	8.98	13.5	40.0	56	20	34		7.5	3.5	0.5	0	86.0	0	180	1
Deer	Wintrobe <sup>122</sup>	6.37	13.9	40.8	46	17	34			27.0	7.0	2.0	53.0	1.0		1
Dog	Ashley and Quast <sup>123</sup>	8.87	18.0	45.8	87	24	35		14.2-5.2							80
	Briner and Walsman <sup>124</sup>	6.48±0.76	13.6±1.8	44.3±1.4	88±8	21±1.8	31±1.4									34
	Lechmaning et al. <sup>125</sup>	7.17	14.1	47.7	67	20	30	7.0								32
	Mayerson <sup>126</sup>	8.48	13.0	38.8	59	20	34		11.2	74.0	2.0		20.0	4.0	821	80
	Norris et al. <sup>127</sup>	8.20	18.1	(Adult)	24				13.2	71.8	6.4		21.8	1.0		38
	Scarborough <sup>115</sup>	7.20±0.60	12.6	(2 to 8 mos. of age)	24			7.0	11.8±5.0	82.5	4.0		33.3	0.2		31
	Wintrobe et al. <sup>117</sup>	7.03±0.66	14.6±1.4	47.2±4.7	68±4	21±1.4	31±1.6			69.0	5.0	0.7	20.0	6.1	400	700±
Elephant	Nirmalan et al. <sup>128</sup>	2.47±0.43	10.24±2.40	34.6±4.84	142±13	41.8±9.9	29.7±7.3		8.76±2.02	34.2±3.4	8.2	0.7	52.8±5.1	6.1±3.1		14
	Simon <sup>129</sup>	2.61±0.43	13.4±0.28	38.2±1.33	126	48	35		10.16±0.73	38.5±1.8	8.4	0.5	51.7±1.2	2.1±0.4		15
Ferrat	Wintrobe <sup>122</sup>	9.98	15.2	51.0	43	18	30									3
Fox	Wintrobe <sup>122</sup>	7.99	12.6	42.4	53	18	30			60.0	7.0	2.0	25.0	6.0		1
Goat	Deerbach <sup>130</sup>	10.70	9.6	30.0	18	8	32									4
	Wintrobe <sup>122</sup>	12.33	11.4	33.2	18	7	38		7.4	38.5	3.5	0	58.0	2.0	2500	2
Guldeer pig	Crastach <sup>121</sup>	8.41	14.2	45.4	71	22	31									8
	King and Lucas <sup>131</sup>	8.08							17.4	31.1*	3.5	0.2	63.4	1.6		9
	Scarborough <sup>115</sup>	8.75±1.20						7.1	10.8	41.8*	4.8	0.8	45.3	8.4		500
	Wintrobe <sup>122</sup>								5.5						250	4
Hamster	Stewart et al. <sup>132</sup>	7.8±0.5	17.9±1.0	47.4±2.4	83	23	37	8.6	8.56±1.54	29.0	0.7		67.9	2.4		316
Horse	Scarborough <sup>115</sup>	8.0-8.5														53
	Kuhnke <sup>119</sup>	8.4-7.4														20
	Vaughan <sup>116</sup>	6.5-8.3	11-15	31.6	52	18	34									3
	Grasnick <sup>133</sup>	6.05	10.7	43.5	42	13	33									5
	Macedo and Ponder <sup>134</sup>	10.35	13.9					5.9-5.3	6.4-8.9							

Jackal	Wintrobe <sup>11</sup>	5.06	12.2	35.5	70	24	34		54.0	5.0	0	41.0					
	Wintrobe <sup>10</sup>	6.52	10.9	38.0	55	17	30		32.0	15.0	0	53.0	1				
Kinkajou	Wintrobe <sup>10</sup>	15.00	14.9	35.9	25	10	40		47.5	1.5	0	50.0	2				
Llama	Knoll <sup>13,14</sup>	15.35							66.0	4.8	4.0	21.9	3.3				
	Ponder et al <sup>15</sup>								57.0	7.4	24.0	7.4	4.3				
Marmoset	Burns et al <sup>16</sup>	6.55±0.63		47.5±5.31					14.37±4.29	63.4±13.9	1.25	0.27	33.9±14.3	2.3±2.5	455±127		
Minik	Trombold <sup>16</sup>	9.00	20.4	56.7	64	24	35		11.8	68.0	0.4	0.2	27.4		598		
Monkey	Hajj <sup>17</sup>	4.94							14.2	42.8	2.8	0.3	52.1	1.2	8		
	Ponder et al <sup>16</sup>	5.50							8.4	71.5	2.5	2.5	22.0	1.5	3		
	Shukla et al <sup>18</sup>	5.20	12.2	40.0	77	24	31		15.1	36.0			59.0		475		
	(4.6-5.8)	(10.9-13.5)	(35-44)						(9.7-20.6)	(20-52)			(44-74)		19		
Wintrobe <sup>10</sup>		5.44	13.2	43.5	84	28	31			23.0	3.0	1.5	70.5	2.0	4		
Mouse	Fehr <sup>19</sup>	7.81		36.7	48				6.6	28.0					44		
	Graslich <sup>18</sup>	9.24	14.3	47.1	51	16	30		8.1	8.5	26.2	2.0	0.5	87.8	7.5	60±	
	Scarborough <sup>17</sup>	9.70±1.70			49	17	35		11.0						270	118	
	Wintrobe <sup>10</sup>	8.88	15.1	43.0													
	Kemeny <sup>18</sup>	8.95															
Opossum	Wintrobe <sup>10</sup>	4.00	10.1	31.9	79	28	32		12.0	39.0	4.7	1.0	46.0	9.3	250	4	
	Jordan <sup>18</sup>	6.90	10.0	35.0					15.3	26.0	3.3	1.0	62.8	7.0		300	
Pig	Wintrobe <sup>11</sup>	7.93	15.0	48.3	58	19	33		5.5	70-20.0							
	Gorge <sup>18</sup>	7.09±	13.0	43.2	60	18	30										
		6.90±	11.8	38.9	57	17	30										
	Scarborough <sup>17</sup>	6.74							8.1	80-20.0	39.0	4.5	1.2	82.1	3.3	215	61
Rebbit	Wintrobe et al <sup>14</sup>	6.28±0.60	13.0±1.5	39.8±4.3	64±4	21±1.6	33±1.7										
	Cassy et al <sup>16</sup>	6.37	11.9%	(nuclei only)					7.7	49.4*	1.5	6.7	32.9	9.8	500	180	
	Scarborough <sup>17</sup>	6.62±1.20			8.7	7.9	43.4*				2.0	4.3	41.8	9.0	800	900±	
Raccoon	Zundel <sup>16</sup>	7.77	11.4	37.6	49	14	30		10.8	47.0			51.0	2.0	285		
Rat	Wintrobe et al <sup>12</sup>	6.60±0.76	13.0±1.2	38.4±3.6	61±4	20±2.1	33±2.3		8.6	40	6.5	0	90.0	5.3	330	73	
	Scarborough <sup>17</sup>	6.50±1.50							8.3	80-15.0	27.0	2.1	0.8	67.9	5.3	800	200±
	Wills and Mehlig <sup>18</sup>	8.55	14.0														
	Cameron and Watson <sup>18</sup>	8.80±	14.6	43.5	52	17	30		5.98	21.4	8-24	0-4	70-89	1-6	673		
	Watson <sup>18</sup>	8.70±	13.8	41.8	49	16	34		6.14	20.4					532		
Sheep	Wintrobe <sup>10</sup>	10.50	12.9	35.7	35	12	35		5.0	28.0	6.0	0	62.0	2.0	350	4	
	Graslich <sup>18</sup>	10.90	11.8	38.5	35	11	31								1		
Skunk	Wintrobe <sup>10</sup>	10.00	15.1	61.4	54	16	30		15.0	46.0	7.0	0	42.0	3.0	540	2	
Sloth	Zundel <sup>16</sup>	2.30	11.0	38.0	165	49	29		21.43	24.0			76.0		389		
Woodchuck	Jordan <sup>18</sup>	7.33	13.9	48.0	66	19	30		7.4	15.7	70.2	1.7	0.4	26.2	0.7	3	

RBC red cell count  $\times 10^{12}/l$  Hgb hemoglobin g/dl blood Ht volume of packed red cells ml/dl MCV mean corpuscular volume in fl (cubic microns) MCH mean corpuscular hemoglobin n pg (micron programs) MCHC mean corpuscular hemoglobin concentration in g/dl diam mean erythrocyte diameter (in dry smears) in  $\mu m$  WBC leukocyte count  $\times 10^9/l$  N neutrophils E eosinophils B basophils L lymphocytes all in %. Pl platelets  $\times 10^9/l$  Values following  $\pm$  in most instances represent  $\pm$  standard deviations

\*In rabbits and in guinea pigs the cells listed under N are pseudo-eosinophils amphiphiils or heterophils. The granules are eosinophilic for the most part rounded and of fairly uniform size but are not as densely packed as in true eosinophils. In these animals the granules in the true eosinophils are very abundant large and ovoid or bluntly fusiform



Fishes		Field et al. <sup>10a</sup>	0.84	10.5	31.3	311	72	34	418±375	0.71	0.04	1.55	88.7	8.31	5.44
Carp	Martins and Pilonberrill <sup>12</sup>	2.75±8.5	8.6±2.61												
Herring	Martins and Pilonberrill <sup>12</sup>	3.48±0.76	11.1±1.60	47.0±5.40					37.89±30.34	1.88	1.25	0.03	84.6	12.3	37.76±21.0
Meckrel	Wintrobe <sup>12a</sup>	4.20	15.2	59.0	140	36	26	12.3×8.6							
Rock cod	Wintrobe <sup>12a</sup>	1.49	5.2	23.8	159	35	22	11.3×8.6							
Rusty flounder	Wintrobe <sup>12a</sup>	0.78	2.1	8.4	108	28	25	10.7×7.3	6.0	2.0		6.0	10.0	84.0	4.0
Trout	Field et al. <sup>10a</sup>	1.01	8.5	27.2	314	75	31								
Dogfish	Wintrobe <sup>12a</sup>	0.07	1.4	7.3	1,010	195	19	22.5×17.1	45.0						
	Reznikoff and Reznikoff <sup>14</sup>	0.39	4.4						83.5	5.0	17.5	0	66.5	1.0	13.6
Skate	Wintrobe <sup>12a</sup>	0.10	1.6												
Teleosts	Srivastava <sup>11a</sup>	1.4-4.3	7.6-14.2	25.6-33.2	60-195	28-107	23-55								
freshwater															
Cyclostomes															
Hagfish	Wintrobe <sup>12a</sup>	0.13	4.2	19.8	1,560	330	21	26.8×18.2	33.0						

\*Average of 14 authors quoted by Macleth and Higgins

†The values given are only a few representative determinations. In the article cited erythrocyte values of a great variety of animals especially fishes are reported

Same as in Table B 1. In addition N refers to polymorphonuclear leukocytes without granules. PEG refers to leukocytes with polymorphonuclear nuclei and eosinophilic granules. PER refers to similar cells with eosinophilic rods. The refers to thrombocytes 10<sup>7</sup>/l.

In birds and in certain selachians and reptiles there is no finely granular special cell corresponding to the polymorphonuclear neutrophil of man and amphibia. Its place is taken by a leukocyte with ellipsoidal or beccary granules.

The differential leukocyte counts recorded above give only an approximate indication of the findings. Full descriptions of cells together with drawings will be found in the excellent monograph by Jordan<sup>10</sup> and in the other references cited at the end of the monograph.

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## APPENDIX C: Blood Dyscrasias Associated with Various Drugs and Chemical Agents

The following table is intended to provide a ready indication regarding the type of dyscrasia that has been reported in association with exposure to the various substances listed. Details and references will be found in the chapters dealing with these dyscrasias.

For:

granulocytopenia, see Table 41-7, page 1292

hemolytic anemia, see Table 27-7, page 918

megaloblastic anemia, see Chapter 14 and Table 14-3, page 574

methemoglobinemia, see Table 31-1, page 1012

pancytopenia, see Table 56-2, page 1746

porphyria, see Tables 32-3, 32-4, pages 1026 and 1035

sideroblastic anemia, see Table 18-4, page 686

thrombocytopenia, see Table 34-5, page 1084

For chemotherapeutic agents and their effects on the hematopoietic system, see Chapter 55.

++++ indicates a substantial number of reported cases, + only a rare report, ++ and +++ are intermediate between these extremes.

+<sup>1</sup> in the pancytopenia column indicates "pure red cell" aplasia.

+<sup>2</sup> in the porphyria column indicates "in animal studies or liver cell culture."

Name of Drug	Anemia			Granulocytopenia	Methemoglobinemia	Pancytopenia	Porphyria	Thrombocytopenia
	Hemolytic	Megaloblastic	Sideroblastic					
acetanilide				++	++++			
acetazolamide				+		+		++
acetaminophen								+
Acetophenetidin				++				
acetylsalicylic acid (aspirin)				+		+ <sup>1</sup>		+
alcohol		++	+				++	++
Aldomet	+++							
Allonal				+				
Allonal (new)				++				
allylisopropyl acetyl carbamide							+ <sup>2</sup>	++++
allylisopropyl barbituric acid				++				+
Allymid								+
alpha methyl dopa	+++							

Name of Drug	Anemia			Granulocytopenia	Methemoglobinemia	Pancytopenia	Porphyria	Thrombocytopenia
	Hemolytic	Megaloblastic	Sideroblastic					
Amidophen				+++				
aminophenol					++			
aminopyrine				++++			++	+
aminosalicylic acid	++					+		++
amobarbital								+
amphotericin B						+		
ampicillin				+				
amyl nitrite					+++			
Amytal Compound				++++				
aniline	++							
aniline dyes					++++			
Anisabuse								+
antizoline								++
antimony				+				
antipyrine				++++				
arsenicals inorganic				++++		++		++
arsenicals organic				+++		++	++	+++
arsphenamina						+		
Atabrine						++++		
Azulfidine				++				
barbiturates				+			++++	
bemegride							+	
benzene	++			+++		++		+++
benzocaine (suppositories)					++			
bismuth						+		+
butabarbital						+		+
Butazolidin				+++				
carbamazepine						+		++
carbamazole				++		++		
carbon tetrachloride						+		
carbromal							+	
carbutamide				+++		+		+
Causalin				++++				
Celoniin							++	
centlin								++
cephalosporin derivatives	++							

Name of Drug	Anemia			Granulocytopenia	Methemoglobinemia	Pancytopenia	Porphyria	Thrombocytopenia
	Hemolytic	Megaloblastic	Sideroblastic					
cephalothin				+				+
chenopodium						+ <sup>1</sup>		
chlorpheniramine maleate								+
chloral derivatives							++	
chloramphenicol			++	++		++++	+ <sup>2</sup>	+
chlordan						+	+ <sup>2</sup>	
chlorthiazepoxide						+		
Chloromycetin			++	++		++++	+ <sup>2</sup>	+
chlorophenothane						+		
chloroquine								+
chlorothiazides								++
chlorpromazine	++			++++		+		+
chlorpropamide				+		+ + <sup>1</sup>	++	++
chlortetracycline						+		
chlorthalidone				+				
chlorthiazides				+				
Cibalgin				++++				
cincophen				+				
codeine								+
colchicine		+ <sup>2</sup>				+ <sup>1</sup>		
cold wave				+				
colloidal silver						+		+
Compazine				++				
contraceptive pills		+					++	
copper sulfate								+
Corosedine				++++				
cycloserine			+					
dapsone				+				
DDT				+				
Demerol								+
desipramine								++
dextroamphetamine sulfate								+
Diabinese				+				
Diamox				+		+		++
Diatrin				+				
diazoxide								+

Name of Drug	Anemia			Granulocytopenia	Methemoglobinemia	Pancytopenia	Porphyria	Thrombocytopenia
	Hemolytic	Megaloblastic	Sideroblastic					
dichlorovinylcysteine						++		
diethazine				+				
diethyl dihydrocollidine							+ <sup>2</sup>	
digitalis								+
digitoxin								++
Dilantin		+++		+		+++ <sup>+1</sup>	++	++
dinitrophenol				+		+		+
diphenylhydantoin sodium		+++		+		+++ <sup>+1</sup>	++	++
dipyrene	++			++++				
disulfiram								+
Diuril				+				
Doriden							+ <sup>2</sup>	
dyes					++	+		
Equanil				+				
ergot								+
erythromycin								+
estrogens		+				++	++	+
ethacrynic acid				+				
ethanol		++	+				++	++
ethinamate							+ <sup>2</sup>	
ethosuximide						+		
ethyl allyl acetylurea								+
S-ethyl phenylhydantoin								+
Flagyl				+				
Furadantin				+				
Gantrisin				+		+		+
gold salts				+++		++		+++
glutethimide							+ <sup>2</sup>	
griseofulvin							++	
hair dyes						++		+
heparin						+ <sup>1</sup>		+
hexachlorobenzene							++++	
hydroxychloroquine								++
hydroxydione							+ <sup>2</sup>	
Hygroton				+				

Name of Drug	Anemia			Granulocytopenia	Methemoglobinemia	Pancytopenia	Porphyria	Thrombocytopenia
	Hemolytic	Megaloblastic	Sideroblastic					
imipramine				++				
indomethacin				++		+		
insecticides	++					+		+
ipanoic acid								+
isoniazid (INH)	++		+			+ <sup>1</sup>	+ <sup>2</sup>	+
Keflin				+				
Kynex				+		+		+
lead	++		++				++	
Librium						+	+ <sup>2</sup>	
Lindane							+ <sup>2</sup>	
mefenamic acid	++							
Mellari				++				
mepazine				++		+		
meperidine								+
meprobamate				+		+	++	+
mercurial diuretics				+				+
mercury						+		
Mesantoin		+++		+		+++	++	
metapyrone							+ <sup>2</sup>	
methaphenilone				+				
methicillin				+		+		
methimazole				++				
methyl dopa	+++						++	++
methylphenylethyl hydantoin				+		+++		
methylphenylhydantoin						++		
methypylon							+ <sup>2</sup>	
metronidazole				+				
Migesic				++++				
Milontin							++	
Miltown				+				
Mysoline		++						
naphthalene					+++			
neomycin		+7						
Neonal compound				++++				
Neostibosan				+				

Name of Drug	Anemia			Granulocytopenia	Methemoglobinemia	Pancytopenia	Porphyria	Thrombocytopenia
	Hemolytic	Megaloblastic	Sideroblastic					
Neurodyne				++++				
nikethamide							+ <sup>2</sup>	
Nirvanol								+
nitrates bismuth ammonium silver					+++			
nitrites amyl sodium					++++			
nitrobenzenes					++			
nitrofurantoin				+				
nitroglycerine					++		+ <sup>2</sup>	+
Noludar							+ <sup>2</sup>	
Novaldin				++++				
Novalgin				++++				
novobiocin				+				++
Nuvarone						+		
Optelidon				++++				
Orinase				+				
oxyphenbutazone				++				+
oxytetracycline						+		+
Pacitol				+				
para aminobenzoic acid				+				
para aminosalicylic acid	++							++
paramethadione								+
parathion						+		
penicillamine				+				
penicillin	+++					+		+
pentachlorophenol						+		
Peralga				++++				
phenacetin	+++			++	++++			+
phenacimide						+		
phenformin		+						
phenindione				+				
phenobarbital		++						+
phenobarbitone							++	
phenols polychlorinated (eg 2,4D)							+	
phenothiazines				++				
phenylbutazone				++++		++		+

Name of Drug	Anemia			Granulocytopenia	Methemoglobinemia	Pancytopenia	Porphyria	Thrombocytopenia
	Hemolytic	Megaloblastic	Sideroblastic					
phenylendiamine					++			
Phencherone						+		
phethenylate				+				
plasmochin				+				
potassium chlorate					++			
potassium chloride		+						
potassium iodide								+
potassium perchlorate						++		
prednisone								+
Presidone				+				
prilocaine					++			
primidone		++						
probenecid							+	
procainamide				+				
prochlorperazine				++				+
progesterone							++	
promazine				++++		+		
promethazine								+
Pronestyl				+				
Prontosil					++			
propylthiouracil				++		+		+
Pyralgin				++++				
Pyramidon	++			++++				
Pyraminol				++++				
pyrazinamide			+			+	+	+
pyrazinoic acid			+					
Pyribenzamine				+		+		
pyrithyldione				+				
quinacrine						++		
quinidine	++							++++
quinine	++			+				++
quinones					+++			
rauwolfia				+				
reserpine								+
resorcin					++			
rifampin								++



[illegible]

Name of Drug	Anemia			Granulocytopenia	Methemoglobinemia	Pancytopenia	Porphyria	Thrombocytopenia
	Hemolytic	Megaloblastic	Sideroblastic					
tetracycline		+						+
tetraethylammonium								+
thematidine				+				
thiocyanate						+		
thioglycolic acid				+				
thiondazone				++				
thiosemicarbazone				+				
thiouracil				+++				
thiourea								+
Thorazine				+++++				
Tibione				+				
Tofranil				++				
tolbutamide				+		+, + <sup>1</sup>	++	++
toluene						++		
toluene di-isocyanate								+
toluenediamine					++			
Tridione				+		+++	+ <sup>2</sup>	
trimethadione				+		+++	+ <sup>2</sup>	+
trimethyloxazolidine				+				
trinitrotoluene					++	++		
Trional							++	
tripelennamine				+		+		
turpentine								+
Valmid							+ <sup>2</sup>	
Veramon				++++				
Veropyron				++++				
Yeast vife				++++				
Zarontin						+		

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